mucopolysaccharides; **proteoglycans**
(separation of unsaturated disaccharide uronic acids of, by t.l.c.)

ST **proteoglycan** colitis
IT Intestine, disease
(colitis; effect of **proteoglycan** on exptl. colitis)
IT Salmon
(composition of **proteoglycan** from salmon head cartilage and effect on exptl. colitis)
IT Hexosamines
Uronic acids
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(composition of **proteoglycan** from salmon head cartilage and effect on exptl. colitis)
IT Glycosaminoglycans, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(composition of **proteoglycan** from salmon head cartilage and effect on exptl. colitis)
IT **Proteoglycans**, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(composition of **proteoglycan** from salmon head cartilage and effect on exptl. colitis)
IT 14808-79-8, Sulfate, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(composition of **proteoglycan** from salmon head cartilage and effect on exptl. colitis)

L15 ANSWER 20 OF 20 ANABSTR COPYRIGHT 2004 RSC on STN
AN 47(4):D116 ANABSTR
TI Determination of unsaturated glycosaminoglycan [mucopolysaccharide] disaccharides by spectrophotometry on thin-layer chromatographic plates.
AU Saamanen, A.-M.; Tammi, M. (Dept. Anat., Univ. Kuopio, 70211 Kuopio 21, Finland)
SO Anal. Biochem. (1984) 140(2), 354-359
CODEN: ANBCA2 ISSN: 0003-2697
DT Journal
LA English
AB Aqueous solution of the mucopolysaccharides or of **proteoglycans** **extracted** from **cartilage** were hydrolysed with hyaluronate lyase at pH 6.2, and proteins and undegraded material were precipitated with ethanol. After centrifugation, the supernatant liquid was evaporated at room temperature in an air stream, and the residue was dissolved in H2O and subjected to t.l.c. on cellulose. Applied samples were desalted by development with butanol - ethanol - H2O (13:8:4), then the unsaturated disaccharides were separated by development for 18 cm with anhydrous **acetic acid** - butanol - aqueous 2M-NH3 (3:2:1) and detected by reflectance scanning at 232 nm. The method was suitable for the range 0.2 to 20 μg of the disaccharide uronic acids. In the range 0.9 to 7 μg, the coefficient of variation (n = 11 to 14) was 15 to 22% on different plates or <10% if samples and standards were on the same plate.
AB Aqueous solution of the mucopolysaccharides or of **proteoglycans** **extracted** from **cartilage** were hydrolysed with hyaluronate lyase at pH 6.2, and proteins and undegraded material were precipitated with ethanol. After centrifugation, the. . . with butanol - ethanol - H2O (13:8:4), then the unsaturated disaccharides were separated by development for 18 cm with anhydrous **acetic acid** - butanol - aqueous 2M-NH3 (3:2:1) and detected by reflectance scanning at 232 nm. The method was suitable for the. . .
IT Matrix:

CLMEN 1 A composition from shark cartilage comprising **proteoglycan** and an immunoactive component having a molecular weight greater than about 100 KD, wherein the 5 composition has a uronic acid content. . .

9 The composition according to any one of claims I to 8, wherein the immunoactive component comprises 5 **proteoglycan**.

L15 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:490169 CAPLUS
DN 137:277009
TI Effect of **proteoglycan** on experimental colitis
AU Majima, Mitsuo; Takagaki, Keiichi; Sudo, Shin-ichiro; Yoshihara, Syuichi; Kudo, Yoshiaki; Yamagishi, Shohei
CS Kakuhiro Co. Ltd., Aomori, 030-8543, Japan
SO International Congress Series (2001), 1223(New Developments in Glycomedicine), 221-224
CODEN: EXMDA4; ISSN: 0531-5131
PB Elsevier Science B.V.
DT Journal
LA English
AB The effect of **proteoglycan** (PG) on colitis was examined in animal expts. using mice. The PG used was **extd.** from nasal **cartilage** of salmon head with 4% **acetic acid** and prepared by precipitation with ethanol followed by dialysis. The PG contained about 7% protein, and had a mol. mass of 344 kDa on SDS/PAGE. The glycosaminoglycan (GAG) sugar chains of the PG were composed of hexosamine, uronic acid and sulfate at a molar ratio of 1.0:1.0:0.7. The mice were divided into a control group and an administration group. The control group was given free access to drinking water containing dextran sulfate sodium salt (DSS) to induce colitis. On the other hand, the administration group was given free access to drinking water containing DSS and PG. Then, the time course of survival rates in both groups were measured. In the administration group, the survival rate increased significantly in comparison with that of the control group. The difference in the survival rates indicated that the onset of mouse colitis induced by DSS was inhibited by administration of the PG.
RE.CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI Effect of **proteoglycan** on experimental colitis
AB The effect of **proteoglycan** (PG) on colitis was examined in animal expts. using mice. The PG used was **extd.** from nasal **cartilage** of salmon head with 4% **acetic acid** and prepared by precipitation with ethanol followed by dialysis. The PG contained about 7% protein, and had a mol. mass of 344 kDa on SDS/PAGE. The glycosaminoglycan (GAG) sugar chains of the PG were composed of hexosamine, uronic acid and sulfate at a molar ratio of 1.0:1.0:0.7. The mice were divided into a control group and an administration group. The control group was given free access to drinking water containing dextran sulfate sodium salt (DSS) to induce colitis. On the other hand, the administration group was given free access to drinking water containing DSS and PG. Then, the time course of survival rates in both groups were measured. In the administration group, the survival rate increased significantly in comparison with that of the control group. The difference in the survival rates indicated that the onset of mouse colitis induced by DSS was inhibited by administration of the PG.

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.
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human and murine haematopoietic progenitor cell lines.

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content and were associated with the highest immunoactivities.

. . .

a non-chromatographed sample within 200-700 nm range) may also be done. It is close to the absorbance range used typically for monitoring **proteoglycans** (206-214 nm) [401. Although only a small local maximum at 280 was observed compared to that of BSA, it was very useful. . .

Proteoglycan determination

Detection of PGs/GAGs was based on the metachromatic shift of the 590 nm peak of DMB solution to 525 nm after complexation. . .

. . .

mitogenic effect on macrophages (i.e. they did not induce macrophage proliferation). The patterns of production of a number of cytokines induced by shark **cartilage extract** activation were examined, and an increase in production of IL-1, IL-G, IL-10, and IL-12 was demonstrated. Figure 8 shows the strong stimulatory effect of shark **cartilage extracts** on macrophage IL-12 and IL-6 production, again demonstrating dose-related responses.

Effect of feeding the shark **cartilage extracts** on susceptibility to infection with LiSteria monocytogenes

Experiments were carried out on the possible protection from infection in vivo, using infection with Listeria monocytogenes. . . number of Listeria colony forming units in the mice fed the different extracts. As shown in Figure 9 we found that the **cartilage extracts** (10 mg dose) tested markedly suppressed the severity of the Listeria infection in terms of the number of bacterial colony forming units found. . .

In addition to buffered guanidine hydrochloride of various strengths, a number of other salts have been used to **extract proteoglycans** from **cartilage**. GuCl is the most popular because the others have very narrow concentration ranges for optimal extraction (321. We did not find any statistical. . .

. . .

a 25% absorbance value compared to that of the BSA standard. Low 5 UV absorbance would suggest either effectively hindered protein cores of **proteoglycans**, a low frequency of aromatic residues, or a low protein content within heterogeneous preparations. Since BCA reacts with a tetradentate-Cu+ complex (produced between. . .

. . .

Lee A, Langer R (1983) Shark cartilage contains inhibitors of tumor angiogenesis. Science 221:1185-7 8. Dupont E, Brazeau P, Juneau C (1997) **Extracts** of shark **cartilage** having an anti-angiogenic activity and an effect on tumor regression: process of making thereof. US

buffer), diluted **acetic acid** and pure water, and may be performed against a membrane having a desired molecular weight cut off. Instead of or in addition. . .

Figure 1A is a graph showing yields of fractions from ultrafiltration of (concentrated) shark **cartilage extract** 3. In Series a, 2 M guanidine hydrochloride was used as the eluent; 64 ml of the solution containing 2.8 g was. . .

Figure 8 is a graph showing stimulation of macrophages by shark **cartilage extract**, determined by release of cytokines IL-6 (0) and IL-12 (0). Production of IL-6 and IL-12 was measured by sandwich ELISA.

Determination of **Proteoglycan** Concentration

Proteoglycan content was determined using DMB as described by Farndale et al[381. Chondroitin sulfate A was employed as the standard.

. . .

of bacteria in the spleens of the control water fed mice and in the spleens of the mice fed with the shark **cartilage extract**.

. . .

splenocytes in vitro. Hot water extractions delivered products in higher yields. Although the products obtained from guanidine based media extractions had higher proportions of **proteoglycans**, as seen from the uronic acid and aminosugar contents as well as from their interaction with DMB, they displayed comparable immunoactivities with. . . produced extracts in vitro as tested on undifferentiated spleen cells are shown in Table 1. Table I provides the yield, the index of **proteoglycan** content, the uronic acid content and immunoactivity in vitro of shark **cartilage extracts** produced by Methods A, B and C. The index of **proteoglycan** content is defined as the ratio of the sample's **proteoglycan** content to the **proteoglycan** content of the supernatant of 40% ammonium sulfate precipitation. Uronic acid content is defined as pg glucuronic acid/pg sample material. Immunoactivities are defined as the cell proliferation due to each sample relative to the cell proliferation due to **cartilage extracted** with 2 M GuCl (2). The samples were tested for immunoactivity at a concentration of 125 pg/ml.

method material yield index of uronic immunoactivity
OUI) **proteoglycan** acid -06
content content
A 4.1 0.80 0.10 82.3
B 2 3.7 0.59 0.14 10 0
C 3 9.4 0.37 0.07 44.2
C 4 7.3. . .

Although the yields of the ammonium sulfate based precipitations were compromised, the lyophilized supernatants had the highest glycosaminoglycan/**proteoglycan**

LPS lipopolysaccharide
mAb Monoclonal antibody
MD megadalton
mw Molecular weight
MWCO molecular weight cut-off
MOPS 3-(N-morpholino)-propane sulfonic acid
Na2EDTA ethylene tetraacetic acid disodium salt
NK natural killer
PG **proteoglycan**
PBS phosphate buffered saline
PMSF Phenylmethanesulfonyl fluoride
PP Peyer's patches
SEC size exclusion chromatography
TBS tris buffered saline
TMB tetramethyl benzidine substrate
TPCK N-tosyl-L-phenylalanine chloromethyl ketone
compositions.

One category of components that make up the shark cartilage immune boosting preaparation are **proteoglycans**.

Proteoglycans are a class of glycosylated proteins, which have covalently linked sulfated glycosaminoglycans, (i.e., chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate). Glycosaminoglycans. . .

Uronic acid is a component of **proteoglycans**. In shark cartilage compositions of the present invention, the 25 uronic acid content is preferably from about 0.05 to about 0.5 gg glucuronic. . .

Proteoglycan content of shark cartilage compositions of the present invention may be measured as the 30 index of **proteoglycan** content, which is defined as the ratio
of the composition's **proteoglycan** content to the **proteoglycan** content of the supernatant of 40% ammonium sulfate precipitation. The index of **proteoglycan** content is preferably from about 0.1 to about 1.0, more particularly from about 0.3 to about 0
While it is believed that the immunoactive components of the composition are **proteoglycans**, the immunoactive components may be different from **proteoglycans** or may be a combination of **proteoglycans** and other component of the composition.

Shark cartilage compositions of the present invention are typically prepared from shark **cartilage** by **extraction** methods. Generally, compositions are prepared by extracting the active immunomodulating principles from shark **cartilage** into an **extraction** medium, separating unwanted
solid materials from the extract, and lyophilizing or freeze-drying the extract to yield solid shark cartilage composition.

.
may be done
against any number of different aqueous media including, but not limited to, diluted alcohols, various buffers (e.g. Tris

[221. McGuire et al demonstrated antiproliferative activity of a heat stable, <10 KD fraction of shark **cartilage extract** on endothelial cell population [4]. This is in agreement with Oikawals work, where a substance with an average molecular mass of 1-10 KD. . .

The protein and carbohydrate components of shark **cartilage extracts** come largely from **proteoglycans** (PG) and represent up to 50% of its dry weight [32]. Cartilage also contains collagen and glycosaminoglycans (GAGs), including chondroitins A, B, and. . .

Proteoglycans might be involved in the interaction of primitive, hematopoietic progenitor cells and stromal cells. IL-3 and GM-GSF can be bound by heparan sulfate. PGs form bone-marrow stromal cells or their extracellular matrix and can be presented in a biologically active form of hematopoietic cells. **Proteoglycans** might also be involved in cell adhesion and homing of hematopoietic stem and progenitor cells [34].

Summary of the Invention

There is provided a composition from shark cartilage comprising **proteoglycans** and an immunoactive 15 component having a molecular weight greater than about 100 KD, wherein the composition has a uronic acid content. . .

There is further provided a formulation comprising an immunomodulating effective amount of a composition from shark cartilage containing **proteoglycan** and an immunoactive component having a molecular weight greater than about 100 KD, together with a physiologically acceptable carrier, excipient and/or diluent, wherein. . .

There is yet further provided a use of an immunomodulating effective amount of a composition from shark cartilage containing **proteoglycans** and an immunoactive component having a molecular weight greater than about 100 KD, wherein the composition has a uronic acid content of about. . .

. . .

provided a method comprising administering to a subject in need of immunomodulation an immunomodulating effective amount of a composition from shark cartilage containing **proteoglycan** and an immunoactive component having a molecular weight greater than about 100 KD, wherein the composition has a uronic acid content of about. . .

Still yet further, there is provided a commercial package comprising an immunomodulating effective amount of a composition from shark cartilage containing **proteoglycan** and an immunoactive component having a molecular weight greater than about 100 KD, wherein the composition has a uronic acid content of about. . .

. . .

kilodalton

CLM What is claimed is:
. . . as an inhibitor of cell proliferation comprising the steps of providing connective tissue having a high content of collagen or **proteoglycans** in condition for extraction, extracting said inhibitor from said tissue with an aqueous extraction medium which includes a solute which. . .

L15 ANSWER 18 OF 20 PCTFULL COPYRIGHT 2004 Univentio on STN
AN 2003068249 PCTFULL ED 20030903 EW 200334
TIEN SHARK CARTILAGE EXTRACS AND USE THEREOF FOR IMMUNOMODULATION
TIFR EXTRAIT DE CARTILAGE DE REQUIN ET UTILISATION DE CELUI-CI A DES FINS D'IMMUNOMODULATION
IN KRALOVEC, Jaroslav, A., 11 Berkshire Close, Halifax, Nova Scotia B3S 1H4, CA [CA, CA];
SING, Franck, Chung-Yin, 1 Feldspar Cres., Halifax, Nova Scotia B3R 2K7, CA [CA, CA]
PA OCEAN NUTRITION CANADA LIMITED, 1721 Lower Water Street, Halifax, Nova Scotia B3J 1S5, CA [CA, CA], for all designates States except US;
KRALOVEC, Jaroslav, A., 11 Berkshire Close, Halifax, Nova Scotia B3S 1H4, CA [CA, CA], for US only;
SING, Franck, Chung-Yin, 1 Feldspar Cres., Halifax, Nova Scotia B3R 2K7, CA [CA, CA], for US only
AG KOENIG, Hans, Smart & Biggar, P.O. Box 2999, Station D, 900 - 55 Metcalfe Street, Ottawa, Ontario K1P 5Y6, CA
LAF English
LA English
DT Patent
PI WO 2003068249 A1 20030821
DS W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
RW (ARIPO): GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
RW (EAPO): AM AZ BY KG KZ MD RU TJ TM
RW (EPO): AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
RW (OAPI): BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
AI WO 2002-CA174 A 20020215
ABEN Disclosed is a composition from shark cartilage comprising immunoactive **proteoglycans** and other immunoactive components having a molecular weight greater than about 100 KD, wherein the composition has a uronic acid content of about 0.05-0.5 μg glucuronic acid per μg of the composition. The composition may be useful for treating or preventing tumor growth, bacterial infections, viral infections and/or fungal infections.
ABFR La presente invention concerne une composition de cartilage de requin comprenant des proteoglycanes immuno-actifs et d'autres composants immuno-actifs qui possedent un poids moleculaire superieur a environ 100 KD, cette composition possedant un contenu en acide uronique compris entre environ 0,05μg et 0,5 μg d'acide glucuronique par μg de cette composition. Cette composition peut convenir pour traiter ou prevenir une croissance tumorale, des infections bacteriennes, des infections virales ou des infections fongiques.
ABEN Disclosed is a composition from shark cartilage comprising immunoactive **proteoglycans** and other immunoactive components having a molecular weight greater than about 100 KD, wherein the composition has a uronic acid. . .

DETD . . . shark cartilage: sphyrnastatin I and 2

Table I shows the cell counts of the dishes containing lyophilized **cartilage extract** at three different concentrations of lyophilized extract.

DETD TABLE I

EFFECT OF **CARTILAGE EXTRACT** ON ENDOTHELIAL CELL GROWTH

Dose, micrograms lyophilized extract per ml. of culture medium	Cells/dish
500	52,000
100	285,000
20	510,000
0	700,000

DETD Similar testing was done utilizing the fraction of lyophilized **cartilage extract** having a molecular weight greater than 50,000. There was no observed inhibition of proliferation of endothelial cells using this fraction,. . .

DETD However, the fraction of lyophilized **cartilage extract** having a molecular weight of 50,000 and below was substantially more potent as a growth inhibitor than the lyophilized extract. . .

DETD TABLE II

EFFECT OF **CARTILAGE EXTRACT** HAVING A MOLECULAR WEIGHT OF 50,000 AND BELOW ON ENDOTHELIAL CELL GROWTH

Dose micrograms lyophilized extract per ml. of culture medium	Cells/dish
500	22,000
100	65,000
20	260,000
5	300,000
0. . .	

DETD The same lyophilized **extracts** of bovine **cartilage** used in the tests of which the results are shown in Tables I and II were evaluated for activity in. . . no significant growth inhibition, nor was there significant growth inhibition of steer fibroblasts by lyophilized extracts of dermis. However, lyophilized **extract** from bovine **cartilage** was found to inhibit proliferation of fetal bovine fibroblasts. The inhibitory effect of lyophilized **extract** from bovine **cartilage** on fetal bovine fibroblasts was less marked than the inhibitory effect on bovine endothelial cells. There was also growth inhibition by **extracts** from bovine **cartilage** on infant human foreskin fibroblasts, but again the degree of inhibition was less marked than in the case of endothelial. . .

DETD . . . dermis did not inhibit growth of fetal bovine fibroblasts, although they did inhibit growth of endothelial cells as effectively as **cartilage extracts**. Lyophilized **extracts** of bovine aorta and of canine cartilage also inhibited endothelial cell growth.

medium which includes a solute which. . .

L15 ANSWER 17 OF 20 USPATFULL on STN
AN 77:43353 USPATFULL
TI Preparation of tissue invasion inhibitor and method of treatment utilizing the inhibitor
IN Kuettner, Klaus E., Chicago, IL, United States
Eisenstein, Reuben, Lincolnwood, IL, United States
Sorgente, Nino, Los Angeles, CA, United States
PA Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, United States (U.S. corporation)
PI US 4042457 19770816
AI US 1975-630275 19751110 (5)
DT **Utility**
FS Granted
EXNAM Primary Examiner: Rosen, Sam
LREP Fitch, Even, Tabin & Luedeka
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 318
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A composition of matter having activity as an inhibitor of cell proliferation is obtained by aqueous extraction of tissue having a high content of collagen and/or **proteoglycans**.
DT **Utility**
AB . . . as an inhibitor of cell proliferation is obtained by aqueous extraction of tissue having a high content of collagen and/or **proteoglycans**.
SUMM . . . herein, an inhibitor of cell growth, is prepared by extractive methods from tissue having a high content of collagen and/or **proteoglycans**, and preferably from connective tissue. The extract may be treated so as to concentrate the inhibiting substance. The resultant concentrate. . .
SUMM . . . extracted with an aqueous extraction medium. A preferred extraction medium includes a solute which does not irreversibly denature proteins or **proteoglycans**. One such preferred extraction medium comprises a 1.0-3.0 M aqueous solution of guanidine hydrochloride.
SUMM . . . aqueous extraction medium, with or without agitation, for a period of time sufficient to result in extraction of polypeptides and **proteoglycans** in substantial yield, or until equilibrium conditions are attained. Extraction is desirably effected at temperatures below room temperature, and preferably. . .
DETD There were thereby obtained lyophilized **extracts** of bovine **cartilage** and of bovine dermis, as well as lyophilized **extracts** from bovine **cartilage** which had been fractionated into an extract having a molecular weight of 50,000 or below and an extract having a. . .
DETD . . . cultures were refed on the third day after test materials were added. A 2.25 % solution of disodium ethylene diamine tetra-**acetic acid** was then prepared in Ca-Mg free phosphate buffered saline, pH 7.4. The cells were then removed from the dish by. . .
DETD The effects of the lyophilized **extract** of bovine **cartilages** on the growth of endothelial cells were determined. 40,000 endothelial cells in 2 ml of culture medium were initially dispensed. . .
DETD . . . original population of 40,000 cells had increased to 700,000.

lyophilized extract.

DETD TABLE I

EFFECT OF **CARTILAGE EXTRACTION** ENDOTHELIAL CELL GROWTH	
Dose,	Cells/dish
micrograms lyophilized extract per ml. of culture medium	
500	52,000
100	285,000
20	510,000
0	700,000

DETD Similar testing was done utilizing the fraction of lyophilized **cartilage extract** having a molecular weight greater than 50,000. There was no observed inhibition of proliferation of endothelial cells using this fraction,. . .

DETD However, the fraction of lyophilized **cartilage extract** having a molecular weight of 50,000 and below was substantially more potent as a growth inhibitor than the lyophilized extract. . .

DETD TABLE II

EFFECT OF **CARTILAGE EXTRACT** HAVING A MOLECULAR WEIGHT OF 50,000 AND BELOW ON ENDOTHELIAL CELL GROWTH	
Dose	Cells/dish
micrograms lyophilized extract per ml. of culture medium	
500	22,000
100	65,000
20	260,000

. . .

DETD The same lyophilized **extracts** of bovine **cartilage** used in the tests of which the results are shown in Tables I and II were evaluated for activity in. . . no significant growth inhibition, now was there significant growth inhibition of steer fibroblasts by lyophilized extracts of dermis. However, lyophilized **extract** from bovine **cartilage** was found to inhibit proliferation of fetal bovine fibroblasts. The inhibitory effect of lyophilized **extract** from bovine **cartilage** on fetal bovine fibroblasts was less marked than the inhibitory effect on bovine endothelial cells. There was also growth inhibition by **extracts** from bovine **cartilage** on infant human foreskin fibroblasts, but again the degree of inhibition was less marked than in the case of endothelial. . .

DETD . . . dermis did not inhibit growth of fetal bovine fibroblasts, although they did inhibit growth of endothelial cells as effectively as **cartilage extracts**. Lyophilized **extracts** of bovine aorta and of canine cartilage also inhibited endothelial cell growth.

CLM What is claimed is:

. . . of cell proliferation .Iadd.and tissue invasion .Iaddend.comprising the steps of providing connective tissue having a high content of collagen or **proteoglycans** in condition for extraction, extracting said inhibitor from said tissue with an aqueous extraction

TI Cell proliferation and tissue invasion inhibitor
IN Kuettner, Klaus E., 426-B W. Webster, Chicago, IL, United States 60614
Eisenstein, Reuben, 6708 N. Keating, Lincolnwood, IL, United States 60646
Sorgente, Nino, 1832 Edgecliffe Dr., Los Angeles, CA, United States 90026
PI US 30239 19800325
US 4042457 19770816 (Original)
AI US 1978-912861 19780605 (5)
US 1975-630275 19751110 (Original)
DT **Reissue**
FS Granted
EXNAM Primary Examiner: Rosen, Sam
LREP Fitch, Even & Tabin
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 320
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A composition of matter having activity as an inhibitor of cell proliferation .Iadd.and tissue invasion.Iaddend.is obtained by aqueous extraction of tissue having a high content of collagen and/or **proteoglycans**.
DT **Reissue**
AB . . . inhibitor of cell proliferation .Iadd.and tissue invasion.Iaddend.is obtained by aqueous extraction of tissue having a high content of collagen and/or **proteoglycans**.
SUMM . . . herein, an inhibitor of cell growth, is prepared by extractive methods from tissue having a high content of collagen and/or **proteoglycans**, and preferably from connective tissue. The extract may be treated so as to concentrate the inhibiting substance. The resultant concentrate. . .
SUMM . . . extracted with an aqueous extraction medium. A preferred extraction medium includes a solute which does not irreversibly denature proteins or **proteoglycans**. One such preferred extraction medium comprises a 1.0-3.0 M aqueous solution of guanidine hydrochloride.
SUMM . . . aqueous extraction medium, with or without agitation, for a period of time sufficient to result in extraction of polypeptides and **proteoglycans** in substantial yield, or until equilibrium conditions are attained. Extraction is desirably effected at temperatures below room temperature, and preferably. . .
DETD There were thereby obtained lyophilized **extracts** of bovine **cartilage** and of bovine dermis, as well as lyophilized **extracts** from bovine **cartilage** which had been fractionated into an extract having a molecular weight of 50,000 or below and an extract having a. . .
DETD The cultures were refed on the third day after test materials were added. A 2.25% solution of disodium ethylene diamine tetra-**acetic acid** was then prepared in Ca-Mg free phosphate buffered saline, pH 7.4. The cells were then removed from the dish by. . .
DETD The effects of the lyophilized **extract** of bovine **cartilages** on the growth of endothelial cells were determined. 40,000 endothelial cells in 2 ml of culture medium were initially dispensed. . .
DETD . . . original population of 40,000 cells had increased to 700,000. Table I shows the cell counts of the dishes containing lyophilized **cartilage extract** at three different concentrations of

pH is then adjusted to 2.5-3.0 by adding concentrated **acetic acid** to a final concentration of 0.5M. The acid extraction is continued for 24 (+/-2) hours with agitation.

DETD The homogenized fine particles of articular **cartilage** are **extracted** in 10 volumes of 4M guanidine hydrochloride (GnC1.sub.2) for 24 (+/-2) hours at 4° C. with constant shaking. The extractant containing the **proteoglycans** and other non-collagenous materials is discarded.

L15 ANSWER 15 OF 20 USPATFULL on STN
AN 82:52151 USPATFULL
TI Anti-invasion factor containing cultures
IN Kuettner, Klaus E., Chicago, IL, United States
PA Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, United States (U.S. corporation)
PI US 4356261 19821026
AI US 1980-142731 19800422 (6)
DT **Utility**
FS Granted
EXNAM Primary Examiner: Kepplinger, Esther M.
LREP Fitch, Even, Tabin, Flannery & Welsh
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 323
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Chondrocytes are cultured to produce cartilage tissue from which an anti-invasion factor (AIF) may be recovered. Mammalian cartilage providing at least about 60% viable chondrocytes is enzymatically digested to denude the cells of their extracellular matrix. The denuded cells are plated at high density and cultured to produce cartilage tissue.

DT **Utility**

SUMM Current methods of obtaining AIF involve obtaining waste animal parts from slaughterhouses, separating the **cartilage** tissue and **extracting** the AIF. This is a long and tedious procedure. Cartilage tissue is a part of the skeleton of animals and,. . .

SUMM . . . cells, whether reproductively active or inactive, are generally called chondrocytes. The extracellular matrix, comprised largely of collagen type II and **proteoglycans** is extremely long lasting; cartilage collagen in certain animals may have a half-life of several years. Accordingly, when the chondrocytes. . .

SUMM . . . containing 5% bovine serum for 90 minutes. The pronase will digest most of the proteins of the extracellular matrix including **proteoglycans** and some collagen. The shavings after washing and centrifugation are further digested in medium containing 5% bovine serum and between. . .

DETD . . . the precipitate is collected by centrifugation following an overnight incubation at 4° C. The pellet is dissolved in 0.5 M **acetic acid** containing 1 mg/ml type I carrier collagen and 10 micrograms per ml. pepsin. Digestion is carried out for 10 hours. . . to 25% saturation with ammonium sulfate to precipitate the protein. The pellet produced by centrifugation is dissolved in 0.5 M **acetic acid** and exhaustively dialyzed against the same solution. The collagen, free of labeled, unincorporated amino acid, is lyophilized and subsequently dissolved. . .

L15 ANSWER 16 OF 20 USPATFULL on STN
AN 80:14237 USPATFULL

Li, Shu-Tung, Oakland, NJ, United States
PA ReGen Corporation, San Francisco, CA, United States (U.S. corporation)
PI US 5306311 19940426
AI US 1991-809003 19911217 (7)
DCD 20080416
RLI Continuation-in-part of Ser. No. US 1990-520027, filed on 19 May 1990 which is a continuation-in-part of Ser. No. US 1989-317951, filed on 2 Mar 1989, now patented, Pat. No. US 5007934 which is a continuation-in-part of Ser. No. US 1987-75352, filed on 20 Jul 1987, now patented, Pat. No. US 4880429
DT **Utility**
FS Granted
EXNAM Primary Examiner: Isabella, David
LREP Lappin & Kusmer
CLMN Number of Claims: 27
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 955
AB A prosthetic, resorbable articular cartilage and methods of its fabrication and insertion are disclosed. The prosthetic articular cartilage, when implanted in a humanoid joint, acts as a resorbable scaffold for ingrowth of native articular chondrocytes and supports natural articulating joint forces. The prosthetic articular cartilage is a dry, porous, volume matrix of biocompatible and bioresorbable fibers. These fibers include a natural polymer or analogs thereof, at least a portion of which may be crosslinked. The matrix is adapted to have an in vivo outer surface contour substantially the same as that of natural articular cartilage in an articulating joint, and has a pore size in the approximate range of about 100 microns to about 400 microns.
DT **Utility**
DETD . . . fascia and extraneous tissues and minced. The minced tendon is first extracted in a salt solution at neutral PH. Salt **extraction** of **cartilage** at neutral pH removes a small portion of the collagen molecules that are newly synthesized and have not yet been incorporated into the stable fibrils. Salt also removes some glycoproteins and **proteoglycans** that are associated with collagen through electrostatic interactions. Useful salt solutions contain NaCl, KCl, and the like.
DETD . . . extreme pHs. Both acidic and basic swelling weakens the non-covalent intermolecular interactions, thus facilitating the release of non-covalently attached glycoproteins, **proteoglycans**, and other non-collagenous molecules through the open pores of the collagenous matrices.
DETD . . . SO.sub.4, or the like, reduces the potential risk of denaturation of the collagen. Alkali treatment dissociates the non-crosslinked glycoproteins and **proteoglycans** from the collagen matrices. The alkali also removes the residual lipids through saponification.
DETD The acid swelling may be conducted at a pH below 3 in the presence of **acetic acid**, HCl, or the like. Like the alkali treatment, the acid swelling removes non-crosslinked glycoproteins and **proteoglycans**.
DETD . . . or patellar cartilage. The adhering tissues of the articular cartilage are scraped off with a knife and minced. The minced **cartilage** is **extracted** in a guanidine hydrochloride solution to remove the majority of the cartilage **proteoglycans** . After extensive rinsing with water, the type II collagen rich material is digested with pepsin in an acid solution at. . .
DETD . . . neutralized with HCl and the material is washed with water. The

Collagen fibers are present diffusely throughout the matrix.

DETD . . . vivo cartilage tissue. Interleukin 1β stimulates production of matrix metalloproteases that can degrade cartilage matrix macromolecules and inhibit synthesis of **proteoglycans**. Treatment of the reconstituted tissue with human recombinant interleukin 1β results in a loss of cartilage and matrix components.

DETD . . . μm, were coated with Type I collagen (Vitrogen, Type I collagen, Collagen Corporation), diluted to 1 mg/ml with 12 mM **acetic acid**. Following coating, the inserts were air dried for up to 18 hours and sterilized with ultraviolet light for 15 minutes.

DETD . . . stained with either hematoxylin and eosin to visualize the cells or with the cationic dye, toluidine blue, to stain sulphated **proteoglycans**. In vivo samples of intact cartilage from bovine metacarpophalangeal joints were similarly processed for light microscopy.

DETD . . . are flattened and the chondrocytes in the deep layers are more spherical. Toluidine blue staining shows the presence of sulphated **proteoglycans** throughout the cartilage. The staining is lighter in the superficial zone suggesting there are fewer **proteoglycans** present. Fetal cartilage has no specific cellular organization as is shown in FIG. 3.

DETD . . . having a flattened morphology and a matrix which did not stain with toluidine blue, indicating the relative absence of sulphated **proteoglycans**. Chondrocytes in the mid zone had a less flattened appearance and the matrix contained sulphated **proteoglycans**, as evidenced by the metachromasia produced by toluidine blue staining. Chondrocytes in the deep zone were spherical in appearance and. . .

DETD . . . vivo cartilage tissue. Numerous electron dense granules were present, which appeared as dots throughout the matrix, indicating the presence of **proteoglycans**.

DETD Chondrocyte cultures were established, following the procedures outlined in Example 1 above, and maintained in culture for 2-4 weeks. The **cartilage** tissue was **extracted** with 100 μg/ml of pepsin at 4° C. After 24 hours an additional 100μg of pepsin was added and the. . .

DETD . . . assessed histologically following staining with toluidine blue. Toluidine blue staining was taken as an indication of the presence of sulphated **proteoglycans**. Toluidine blue staining was greatly diminished in cultures treated with IL-1, compared to untreated controls, indicating a loss of cartilage. . .

CLM What is claimed is:

7. A biological material as claimed in claim 1, wherein the cartilage tissue contains **proteoglycans** containing galactosamine and glucosamine having a ratio of galactosamine to glucosamine of 4.5 to 6.5.

8. A biological material as claimed in claim 1, further containing sulphated **proteoglycans** within the matrix.

9. A biological material as claimed in claim 8, wherein the sulphated **proteoglycans** increase in abundance from the superficial to the mid and deep zones.

L15 ANSWER 14 OF 20 USPATFULL on STN
AN 94:35189 USPATFULL
TI Prosthetic articular cartilage
IN Stone, Kevin R., Mill Valley, CA, United States

AI US 1992-835831 19920318 (7)
DT **Utility**
FS Granted
EXNAM Primary Examiner: Green, Randall L.; Assistant Examiner: Nguyen, Dinh X.
LREP Bereskin & Parr
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 616
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to cartilage tissue reconstituted on a substrate; to a method for producing reconstituted cartilage tissue; and to cartilage tissue reconstituted in vitro from isolated chondrocytes cultured on a substrate.

DT **Utility**

SUMM Cartilage is composed of chondrocytes which synthesize an abundant extracellular matrix, which is composed of water, collagens, **proteoglycans** and noncollagenous proteins and lipids. Collagen serves to trap **proteoglycans** and to provide tensile strength to the tissue. Type II collagen is the predominant collagen in cartilage tissue. The **proteoglycans** are composed of a variable number of glycosaminoglycan chains, keratan sulphate, chondroitin sulphate and/or dermatan sulphate, and N-linked and O-linked. . .

SUMM . . . chondrocytes are flattened and lie parallel to the surface embedded in a matrix that contains tangentially arranged collagen and few **proteoglycans**. In the mid zone, chondrocytes are spherical and surrounded by a matrix rich in **proteoglycans** and obliquely organized collagen fibers. In the deep zone, close to the bone, the collagen fibers are vertically oriented. The keratan sulphate rich **proteoglycans** increase in concentration with increasing distance from the cartilage surface (Zanetti et al, supra).

SUMM . . . cultures is labile and the chondrocytes dedifferentiate to fibroblasts, as defined by production of type I collagen and small nonaggregating **proteoglycans** (Von der Mark et al, Nature 267: 531, 1977; and Solursh, Am. J. Med. Gen. 34: 30, 1989).

SUMM . . . conditions the chondrocytes maintain at least a partial chondrocyte phenotype, as indicated by the synthesis of type II collagen and **proteoglycans** specific to articular cartilage. However, there are a number of problems with these types of cultures. Proliferation in some of. . .

SUMM . . . roller bottles. Type II collagen was reported as being the major matrix-associated collagen synthesized in vitro. Kuettner et al analyzed **proteoglycans** synthesized by the chondrocytes in their culture system by chromatography of .sup.35 S pulse-labelled cultures. The **proteoglycans** synthesized were compared with those of in vivo bovine articular cartilage. Bassler et al (1986) teach a suspension culture of human chondrocytes wherein aggregates of chondrocytes with secreted matrix were produced by a gyratory shaker. Type II collagen and **proteoglycans** were detected in the secreted matrix by immunofluoresence and radioimmunoassay.

DETD . . . culture insert, pore size 0.4μm, coated with a growth attachment factor, preferably collagen, most preferably type I collagen diluted in **acetic acid**. The insert may subsequently be air dried and sterilized, for example by ultra violet light.

DETD . . . morphology and a matrix which does not stain, or stains poorly, with toluidine blue, indicating the relative absence of sulphated **proteoglycans**. Chondrocytes in the mid and deep zones have a spherical appearance and the matrix contains abundant sulphated **proteoglycans**, as evidenced by staining with toluidine blue.

Hardingham, Biochem J., 213, pp. 371-78 (1983). In order to prepare the standard "unlabeled" antigen for measuring the epitope recognized by monoclonal antibody 5/6/3-B-3, we pretreated **proteoglycans** isolated from human articular cartilage with chondroitinase ABC. This pretreatment shortens the glycosaminoglycan chain(s) and creates many new carbohydrate chain. . .

DETD . . . with dilute osteoarthritic samples or controls (50 μl) in incubation buffer together with .sup.125 I-labelled chondroitinase ABC digested human cartilage **proteoglycan** (20,000 dpm) in 1.5 ml plastic microcentrifuge tubes and incubated overnight at 20° C. A suspension (10% w/v) of S.. . . standards of known antigen concentration and the results were expressed in units of concentration of the antigen (chondroitinase digested human **proteoglycan**).

DETD Detection of Atypical Chondroitin Sulfate/Dermatan Sulfate Structures In Cartilage **Proteoglycans** from Joints Using Monoclonal Antibodies 5/6/3-B-3 and 8/25/7-D-4

DETD . . . diamine tetracetic acid, benzamidine HCl and 0.001M phenyl-methanesulphonylfluoride). The extract was dialyzed against several changes of 8M urea and the **proteoglycan** concentration was determined in a dye binding assay described by R. W. Farndale et al., Conn. Tiss. Res. 9, pp.. . .

DETD **Proteoglycans extracted** from the **cartilage** from different joint regions were next compared by electrophoresis on composite agarose/acrylamide slab gels. The gels were prepared by suspending. . . cold (4° C.) for 1 hour and then equilibrated in buffer containing 4M urea overnight. Samples containing about 10 μg **proteoglycan** in buffer (0.01M Tris-acetate/0.25 mM Na.sub.2 SO.sub.4 containing 8M urea and 0.001% (w/v) bromophenol blue, pH 6.8) were loaded in. . . mm. Replicate gels were run in this manner. One gel was then stained with toluidine blue (0.2% (w/v) in 0.1M **acetic acid** to reveal **proteoglycan** bands and replicate gels were left unstained for electroblotting.

DETD . . . described above. Similarly, FIG. 2 shows the atypical chondroitin sulfate/dermatan sulfate epitope recognized by 8/25/7-D-4 was expressed more strongly in **proteoglycans** in osteoarthritic cartilage compared with control joints.

DETD Quantitative assessment of the immunoblotting from parallel tracks showed an average increase to 440% of control in the **proteoglycans** from osteoarthritic cartilage (see Table 2).

DETD TABLE 2

8/25/7-D-4 Epitope in **Proteoglycans** from Experimental Canine Osteoarthritis Analysis of Densitometric Scans (FIG. 2)

Animal Number	Region	Epitope Abundance Expressed as % of control Average
74	femoral	428. . .

DETD . . . Example 1, using monoclonal antibody 5/6/3-B-3, except for taking densitometric scans. We observed 11 out of 16 immunoblots of the **proteoglycans** in osteoarthritic samples (FIGS. 4a and 4b) and 3 out of 15 normal samples (FIGS. 3a and 3b) as being. . .

L15 ANSWER 13 OF 20 USPATFULL on STN
AN 94:57402 USPATFULL
TI Reconstituted cartridge tissue
IN Kandel, Rita A., Toronto, Canada
PA Mount Sinai Hospital Corporation, Toronto, Canada (non-U.S. corporation)
PI US 5326357 19940705

Fluorography," Analyt. Biochem, 156, pp. 38-44 (1986), with a composite gel mixture of 1.2% (w/v) polyacrylamide and 0.6% (w/v) agarose. After electrophoresis, the **proteoglycans** are electrophoretically transferred onto nylon sheets and the occurrence of the osteoarthritis markers of this invention are detected by immunolocalization. . .

DETD Monoclonal antibody 5/6/3-B-3 was produced using Swarm rat chondrosarcoma **proteoglycan** core protein as antigen for immunization. The **proteoglycan** core protein was obtained after digestion of the **proteoglycan** monomer, AIDI fraction, with chondroitinase ABC using the conditions described by Christner et al., J. Biol. Chem., 255, 7102-05 (1980). **Proteoglycan** monomer A1D1 was isolated from Swarm rat chondrosarcoma as described by Baker and Caterson, J. Biol. Chem., 254, 2387-2393 (1979).. . . et al., Anal. Biochem., 90, 22-32 (1978). Monoclonal antibody 8/25/7-D-4 was produced using 17 day old embryonic chicken bone marrow **proteoglycan** as antigen. The marrow **proteoglycan** monomer preparation was prepared as described by Sorrell et al., Cell Tissue Res., 252, 523-31 (1988). Femora and tibiae were. . .

DETD Female Balb/cJ mice (Jackson Laboratories; 4-8 weeks old) were immunized using as antigen either Swarm rat chondrosarcoma **proteoglycan** core protein (800 μg/ml) for the monoclonal antibody 5/6/3-B-3 or 17 day old embryonic chicken bone marrow **proteoglycan** monomer (400 μg/ml) for the monoclonal antibody 8/25/7-D-4. The antigen was administered to the mouse at six injection sites (hind foot pads, lateral thoracic and inguinal regions) using a procedure described by Caterson et al., "Biology of **Proteoglycans**" In: Biology of the Extracellular Matrix, T. Wight and R. Mecham, ed., pp. 1-26, Academic Press, N.Y. (1987). Injections were. . .

DETD . . . ml samples of medium from cultures containing hybridomas were taken and analyzed for antibodies recognizing epitopes present on the original **proteoglycan** antigen using ELISA and radioimmunoassay procedures described by Caterson et al., J. Biol. Chem., 258, pp. 8848-54 (1983). Hybridoma cultures. . .

DETD Monoclonal antibody 5/6/3-B-3 was produced with chondroitinase ABC-treated Swarm rat chondrosarcoma **proteoglycan** monomer as an antigen. The antigenic specificity of 5/6/3-B-3 was defined using normal cartilage **proteoglycans** pretreated with either chondroitinase ABC, AC11 or mammalian hyaluronidase. J. R. Couchman et al., Nature, 307, pp. 650-52 (1984); B. Caterson et al., "Monoclonal Antibodies As Probes For Elucidating **Proteoglycan** Structure And Function," In: Biology Of The Extracellular Matrix, ed. T. Wight and R. Mecham, Academic Press, pp. 1-26 (1987).. . . These non-reducing terminal structures containing the 5/6/3-B-3 epitope are present in chondroitin sulfate chains in osteoarthritic human and dog cartilage **proteoglycans** (without prior chondroitinase or hyaluronidase pretreatment) but this epitope is found to a significantly lesser degree in normal or control. . .

DETD Monoclonal antibody 8/25/7-D-4 was produced using 17 day old embryonic chicken bone marrow **proteoglycan** as antigen. In this case, nonchondroitinase treated **proteoglycan** was used as the antigen. Characterization of the specificity of this monoclonal antibody indicated that the 8/25/7-D-4 epitope is only present in **proteoglycans** that have not had their chondroitin sulfate chains extensively cleaved by chondroitinase pretreatment. Exhaustive chondroitinase digestion removes the antigenic determinant. . .

DETD . . . next prepared radiolabelled antigen containing 5/6/3-B-3 epitopes for standardization of our radioimmunoassay ("RIA") by .sup.125 I-iodination of chondroitinase ABC digested **proteoglycans** isolated from human articular cartilage. A. Ratcliffe and T. E.

of **Proteoglycans**, ed., T. Wight and R. Mecham, pp. 1-26, Academic Press, N.Y. (1987). In contrast, it is an essential feature of. . .

DETD Monoclonal antibody 5/6/3-B-3 is available from ICN Biochemical in Cleveland, Ohio, or it may be prepared using Swarm rat chondrosarcoma **proteoglycan** core protein as antigen for immunization. A sample of monoclonal hybridoma 5/6/3-B-3 has been deposited at American Type Culture Collection,. . . 5/6/3-B-3 detects a terminal glucuronic acid residue adjacent to N-acetyl galactosamine-6-sulfate at the non-reducing end of chondroitin sulfate chains in **proteoglycans**.

DETD Monoclonal antibody 8/25/7-D-4 can be produced using 17 day old embryonic chicken bone marrow **proteoglycan** as antigen. In contrast to monoclonal antibody 5/6/3-B-3, enzymatic (chondroitinase or hyaluronidase) cleavage of the chondroitin sulfate/dermatan sulfate chains to. . .

DETD Other antibodies of this invention that are diagnostic for early stages of osteoarthritis are preferably prepared as described above using **proteoglycans** of **cartilage extracts** from a mammal suffering from osteoarthritis as an antigen in standard (conventional) immunization procedures as described in Caterson et al., "Monoclonal Antibodies As Probes For Elucidating **Proteoglycan** Structures and Function," In: Biology of **Proteoglycans**, ed., T. Wight and R. Mecham, pp. 1-26, Academic Press, N.Y. (1987). The antibodies of this invention are then selected by screening the collection of monoclonals produced against these antigens for those (1) that selectively recognize **proteoglycans** of **cartilage extracts** from a mammal suffering from osteoarthritis (preferably the same mammal from which the antigen used to raise the antibody is derived), (2) that do not recognize **proteoglycans** of **cartilage extracts** from a mammal not suffering from osteoarthritis (preferably the same mammal as used in the first screening and more preferably. . . the same mammal as used to derive the antigen and in the first screening) and (3) that do not recognize **proteoglycans** of **cartilage extracts** from a mammal suffering from osteoarthritis, from which the chondroitin sulfate/dermatan sulfate chains have been completely removed by enzymatic or. . .

DETD One such assay, by way of example, is a radioimmunoassay as described by Ratcliffe and Hardingham, "Cartilage **Proteoglycan** Binding Region and Link Protein," Biochem J., 213, pp. 371-78 (1983). In this particular assay system, synovial fluid samples may be analyzed in a competitive radioimmunoassay using .sup.125 I-labelled **proteoglycans** containing the epitope specifically recognized by the monoclonal antibody of this invention as labelled antigen, with heat denatured, formalin fixed S. aureas as an immunoprecipitant. We may then quantitate the results by calibration with a standard **proteoglycan** antigen. In the preferred embodiment of this invention, where 5/6/3-B-3 is the monoclonal antibody, human articular cartilage **proteoglycans** pretreated with chondroitinase would be radiolabelled. For assays using monoclonal antibody 8/25/7-D-4, 17-day old embryonic chicken bone marrow **proteoglycans** would be radiolabelled.

DETD An alternative assay to diagnose osteoarthritis is characterized by the electrophoresis of samples of **cartilage extracts** on composite agarose/acrylamide slab gels. The **extracts** of **cartilage** and composite agarose/acrylamide gels may be, for example, prepared according to the method of Carney et al., "Electrophoresis of .sup.35 S-Labeled **Proteoglycans** on Polyacrylamide-Agarose Composite Gels and Their Visualization by

markers contain subtle structural domains that. . .

DRWD FIG. 1 depicts densitometric scans of 5/6/3-B-3 immunoblots of **proteoglycans** from tibial, femoral and patella groove cartilage from normal and osteoarthritic knee joints of mature dogs 3 months after experimental. . .

DRWD FIG. 2 depicts densitometric scans of 8/25/7-D-4 immunoblots of **proteoglycan** preparations from normal and osteoarthritic cartilage of mature dogs 3 months after experimental osteoarthritis was induced.

DRWD FIGS. 3a and 3b depict densitometric scans of 5/6/3-B-3 immunoblots of **proteoglycans** from normal human cartilage.

DRWD FIGS. 4a and 4b depict densitometric scans of 5/6/3-B-3 immunoblots of **proteoglycans** from osteoarthritic human cartilage.

DETD **Proteoglycans** are major components of the extracellular matrix of cartilage and are responsible for the stiffness and elasticity of the tissue. Cartilage **proteoglycans** are complex macromolecules in which many glycosaminoglycan chains and oligosaccharides are linked to an extended protein core. Five separate domains have been identified in the cartilage **proteoglycan** protein core: a highly folded globular region that forms the hyaluronate-binding site, a second globular domain of unknown function that. . .

DETD . . . chondroitin sulfate/dermatan sulfate chains are those that are specifically recognized by monoclonal antibodies 5/6/3-B-3 (human, dog and guinea pig cartilage **proteoglycans**) or 8/25/7-D-4 (dog and guinea pig cartilage **proteoglycans**). Other such atypical chondroitin sulfate/dermatan sulfate chains are those specifically recognized by the other monoclonal antibodies of this invention.

DETD . . . this invention are characterized by monoclonal antibodies that are selectable using the following protocol: monoclonal antibodies are produced against the **proteoglycans** of **cartilage extracts** from a mammal suffering from osteoarthritis and screened for those (1) that selectively recognize **proteoglycans** of **cartilage extracts** from a mammal suffering from osteoarthritis (preferably the same mammal from which the antigen used to raise the antibody is derived), (2) that do not recognize or that recognize to a significantly lesser extent than in screen (1) **proteoglycans** of **cartilage extracts** from a mammal not suffering from osteoarthritis (preferably the same mammal as used in the first screening and more preferably. . . the same mammal as used to derive the antigen and in the first screening) and (3) that do not recognize **proteoglycans** of **cartilage extracts** from a mammal suffering from osteoarthritis, from which the chondroitin sulfate/dermatan sulfate chains have been completely removed by enzymatic or. . .

DETD In screen (1) and (2) above it is important that **proteoglycans** of **cartilage extracts** from a mammal suffering or not suffering from osteoarthritis are not treated with chondroitinase, hyaluronidase or other enzymes that may. . . treatment with alkaline borohydride, anhydrous hydrogen fluoride or trifluoro methane sulfonic acid. Screen (3) serves to eliminate those antibodies recognizing **proteoglycan** antigens that are not part of chondroitin sulfate/dermatan sulfate chain structures.

DETD Prior to this invention, monoclonal antibody 5/6/3-B-3 had been used to quantify normal chondroitin-6-sulfated **proteoglycans**. This use of monoclonal antibody 5/6/3-B-3 requires that the tissue or sample be pretreated with chondroitinase or hyaluronidase thereby cleaving. . . glycosaminoglycan chains to leave shortened chains characterized by the 5/6/3-B-3 epitope. Caterson et al., "Monoclonal Antibodies As Probes For Elucidating **Proteoglycan** Structures and Function," In: Biology

DETD The acid swelling may be conducted at a pH below 3 in the presence of **acetic acid**, HCl, or the like. Like the alkali treatment, the acid swelling removes non-crosslinked glycoproteins and **proteoglycans**.

DETD . . . or patellar cartilage. The adhering tissues of the articular cartilage are scraped off with a knife and minced. The minced **cartilage** is **extracted** in a guanidine hydrochloride solution to remove the majority of the cartilage polysaccharides. After extensive rinsing with water, the Type. . . shaking at 4° C. Both inorganic acids and organic acids may be used, including hydrochloric acid, phosphoric acid, lactic acid, **acetic acid**, and the like. The pepsin-solubilized collagen is purified by repeated precipitation and redissolution by methods well known in the art.

DETD . . . neutralized with HCl and the material is washed with water. The pH is then adjusted to 2.5-3.0 by adding concentrated **acetic acid** to a final concentration of 0.5M. The acid extraction is continued for 24 (+/-2) hours with agitation.

DETD The homogenized fine particles of articular **cartilage** are **extracted** in 10 volumes of 4M guanidine hydrochloride (GnCl.sub.2) for 24 (+/-2) hours at 4° C. with constant shaking. The extractant containing the **proteoglycans** and other non-collagenous materials is discarded,

L15 ANSWER 12 OF 20 USPATFULL on STN

AN 94:110673 USPATFULL

TI Methods of and compositions for diagnosis, monitoring and treating the early stages of osteoarthritis

IN Caterson, Bruce, Apt. J2, Carolina Apts., 401 Hwy. 54 Bypass, Carrboro, NC, United States 27510
Hardingham, Timothy E., 55 Lambton Road, London SW20, England

PI US 5374529 19941220

AI US 1991-789404 19911101 (7)

RLI Continuation of Ser. No. US 1988-261148, filed on 24 Oct 1988, now abandoned

DT **Utility**

FS Granted

EXNAM Primary Examiner: Kepplinger, Esther M.; Assistant Examiner: Wolski, Susan C.

LREP Haley, Jr., James F., McDonell, Leslie A.

CLMN Number of Claims: 1

ECL Exemplary Claim: 1

DRWN 6 Drawing Figure(s); 6 Drawing Page(s)

LN.CNT 717

AB Methods of and compositions for early diagnosis, monitoring and treatment of osteoarthritis using monoclonal antibodies which specifically recognize antigenic determinants on atypical chondroitin sulfate/dermatan sulfate glycosaminoglycan chains in body tissues and fluids, which determinants are markers of osteoarthritis.

DT **Utility**

SUMM . . . treatment of osteoarthritis using monoclonal antibodies which specifically recognize antigenic determinants on atypical chondroitin sulfate/dermatan sulfate glycosaminoglycan chains of cartilage **proteoglycans** from humans and animals with osteoarthritis, such atypical chains being markers of osteoarthritis.

SUMM . . . these objects by demonstrating for the first time that atypical chondroitin sulfate or dermatan sulfate glycosaminoglycan chains of osteoarthritic cartilage **proteoglycans** are markers for and characterize the early stages of osteoarthritis. We believe that these

The **proteoglycan** decorin binds C1q and inhibits the activity of the C1 complex. J. Immunol. (in press).

L15 ANSWER 11 OF 20 USPATFULL on STN
AN 97:35731 USPATFULL
TI Prosthetic articular cartilage
IN Stone, Kevin R., Mill Valley, CA, United States
Li, Shu-Tung, Oakland, NJ, United States
PA ReGen Biologics, Inc., Redwood City, CA, United States (U.S. corporation)
PI US 5624463 19970429
AI US 1994-232743 19940425 (8)
RLI Continuation of Ser. No. US 1991-809003, filed on 17 Dec 1991 which is a continuation-in-part of Ser. No. US 1990-520027, filed on 7 May 1990 which is a continuation-in-part of Ser. No. US 1989-317851, filed on 2 Mar 1989 which is a continuation-in-part of Ser. No. US 1987-75352, filed on 20 Jul 1987
DT **Utility**
FS Granted
EXNAM Primary Examiner: Isabella, David
LREP Fish & Richardson P.C.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 962
AB A prosthetic, resorbable articular cartilage and methods of its fabrication and insertion are disclosed. The prosthetic articular cartilage, when implanted in a humanoid joint, acts as a resorbable scaffold for ingrowth of native articular chondrocytes and supports natural articulating joint forces. The prosthetic articular cartilage is a dry, porous, volume matrix of biocompatible and bioresorbable fibers. These fibers include a natural polymer or analogs thereof, at least a portion of which may be crosslinked. The matrix is adapted to have an in vivo outer surface contour substantially the same as that of natural articular cartilage in an articulating joint, and has a pore size in the approximate range of about 100 microns to about 400 microns.
DT **Utility**
DETD . . . and in the range of about 0 to 10% in the lower stress areas. However, when uniform, the dispersion of **proteoglycan** throughout the prosthetic articular cartilage may be, for example, in the range of about 1 to 25%.
DETD . . . fascia and extraneous tissues and minced. The minced tendon is first extracted in a salt solution at neutral pH. Salt **extraction** of **cartilage** at neutral pH removes a small portion of the collagen molecules that are newly synthesized and have not yet been incorporated into the stable fibrils. Salt also removes some glycoproteins and **proteoglycans** that are associated with collagen through electrostatic interactions. Useful salt solutions contain NaCl, KCl, and the like.
DETD . . . pHs. Both acidic and basic swelling weakens the non-covalent intermolecular interactions, thus facilitating the release of non-covalently attached glycoproteins, glycosaminoglycans (**proteoglycans**), and other non-collagenous molecules through the open pores of the collagenous matrices.
DETD . . . SO.sub.4, or the like, reduces the potential risk of denaturation of the collagen. Alkali treatment dissociates the non-crosslinked glycoproteins and **proteoglycans** from the collagen matrices. The alkali also removes the residual lipids through saponification.

residual C1 activity as described by Borsos and Rapp (69).

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DETD . . . A. Carrino, M. A. Baber and A. I. Caplan. 1991. Generation of a monoclonal antibody against avian small dermatan sulfate **proteoglycan**: Immunolocalization and tissue distribution of PG-II (decorin) in embryonic tissues. Matrix 11:412-427.

DETD . . . Choi, L-H Tang, T. L. Johnson, S. Pal, C. Webber, A. Reiner and R. Poole. 1985. Isolation of dermatan sulfate **proteoglycans** from mature bovine articular cartilages. J. Biol. Chem. 260:6304-6313.

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DETD 49. Fisher, L. W., J. D. Termine and M. F. Young. 1989. Deduced protein sequence of bone small **proteoglycan** I (biglycan) shows homology with **proteoglycan** II (decorin) and several nonconnective tissue proteins in a variety of species. J. Biol. Chem. 264:4571-4576.

DETD 50. Blochberger, T. C., J-P Vergnes, J. Hempel and J. R. Hassell. 1992. cDNA to chick lumican (corneal keratan sulfate **proteoglycan**) reveals homology to the small interstitial **proteoglycan** gene family and expression in muscle and intestine. J. Biol. Chem. 267:347-352.

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DETD . . . Robenek, B. Harach, J. Glessl, V. Nolte, H. Hormann, H. Richter and H. Kresse. 1987. Interaction of small dermatan sulfate **proteoglycan** from fibroblasts with fibronectin. J. Cell Biol. 104:1683-1691.

DETD 59. Yamaguchi, Y., D. M. Mann and E. Ruoslahti. 1990. Negative regulation of transforming growth factor-b by the **proteoglycan** decorin. Nature 346:281-284.

DETD 60. Brown, D. C. and K. G. Vogel. 1989. Characteristics of the in vitro interaction of a small **proteoglycan** (PGII) of bovine tendon with type 1 collagen. Matrix 9:468-478.

DETD . . . Bidanset, D. J., C. Guidry, L. C. Rosenberg, H. U. Choi, R. Timpl and M. Hook. 1992. Binding of the **proteoglycan** decorin to collagen type VI. J. Biol. Chem. 267:5250-5256.

DETD . . . U. Choi, L. C. Rosenberg, L. Zardi and L. A. Culp. 1987. Fibronectin-mediated adhesion of fibroblasts: Inhibition by dermatan sulfate **proteoglycan** and evidence for a cryptic glycosaminoglycan-binding domain. J. Cell Biol. 105:1443-1454.

DETD . . . G., M. Paulsson and D. Heinegard. 1984. Specific inhibition of type I and type II collagen fibrillogenesis by the small **proteoglycan** of tendon. Biochem. J. 223:587-597.

DETD . . . U., T. L. Johnson, S. Pal, L-H Tang, L. Rosenberg and P. J. Neame. 1989. Characterization of the dermatan sulfate **proteoglycans**, DS-PGI and DS-PGII, from bovine articular cartilage and skin isolated by octyl-sepharose chromatography. J. Biol. Chem. 264:2876-2884.

DETD . . . Funderburgh, J. L., M. L. Funderburgh, M. M. Mann and G. W. Conrad. 1991. Arterial lumican--Properties of a corneal-type sulfate **proteoglycan** from bovine aorta. J. Biol. Chem. 266(36):24773-24777.

DETD 103. Krumdieck, R., M. Hook, L. C. Rosenberg and J. E. Volanakis. 1992.

followed by equilibrium density gradient centrifugation, DEAE-SEPHACEL.TM. chromatography, and gel chromatography on SEPHAROSE.TM. CL-4B. DSPG-I and. . .

DETD Assessing the Biological Action of Structurally Related **Proteoglycans**

DETD Decorin belongs to a family of structurally related matrix **proteoglycans** which have as common features a broad tissue distribution and tandemly repeated leucine-rich motifs which comprise the majority of the primary structures of their core proteins. These **proteoglycans** include biglycan (49), lumican (50,100), and fibromodulin (51). The inventors propose that, some of these structurally related **proteoglycans** also may modulate complement activity. Specifically, the inventors propose to examine whether other members of the decorin family such as. . .

DETD . . . then we will proceed to further characterize the interaction. To determine if the interaction is saturable, increasing amounts of radiolabeled **proteoglycan** of known specific activity will be added to plastic wells coated with C1q, or as a measure of non-specific binding,. . . removed by washing, and bound and unbound radioactivity will be quantitated. Specific binding will be calculated as the amount of **proteoglycan** bound to C1q minus that bound to BSA.

DETD . . . reciprocal of the slope of the line of the Scatchard plot (101). To determine if binding is mediated by the **proteoglycan** core protein or the glycosaminoglycan (GAG) chains, the intact **proteoglycan**, its core protein and GAG chains can be tested as competitive inhibitors of the binding of radiolabeled **proteoglycan** to C1q. To localize the **proteoglycan** binding site of C1q, proteolytic fragments of the collagenous and globular domains of C1q will be tested as competitive inhibitors of the binding of radiolabeled **proteoglycan** to C1q.

DETD Lastly, if either of the two **proteoglycans** bind C1q, we will examine whether they activate or inhibit C1. Activation of C1 will be determined indirectly by measuring C4 consumption following incubation of normal human serum (NHS) with increasing concentrations of the **proteoglycan** or as a positive control, BSA anti-BSA complexes. Inhibition of C1 will be examined by incubating the **proteoglycan** with purified C1 or C1 in NHS, and measuring residual C1 activity by a hemolytic assay. Again, the studies can. . .

DETD Core protein preparations of the **proteoglycans** will be obtained by digesting the intact **proteoglycans** with chondroitinase ABC (0.2 units/mg protein) and the core proteins will be purified by FPLC on a Mono-Q column as described (103). Binding of radiolabeled **proteoglycan** to solid-phase C1q, or of radiolabeled C1q to solid-phase **proteoglycan** will be performed exactly as described in the inventors' manuscript (103). Briefly, IMMULON.TM.-2 microtiter wells will be coated with C1q. . . C. Nonspecific binding sites will be blocked with 1% BSA followed by the addition of 5+10.sup.4 cpm of .sup.125 I-labeled **proteoglycan** in PBS. The mixtures will be incubated at 37° C. for 1-2 hours. Following incubation unbound material will be removed. . . Triton X-100 and bound material will be quantitated in a gamma counter. In some experiments, wells will be coated with **proteoglycan** and radiolabeled C1q will be added.

DETD C4 consumption assay will be performed by incubating aliquots of NHS with increasing concentrations of **proteoglycan** and measuring residual C4 activity according to the method of Gaither (68). Hemolytic units will be calculated as described (104).. . . NHS (diluted to a C1 activity of 1 unit/ml in low ionic strength veronal buffered saline) with increasing concentrations of **proteoglycan**, and measuring

proteoglycan decorin has the ability to bind to C1q and suppress or inhibit C1 complex biological activity. In diseases where complement. . . advantages over existing therapies and an ideal solution to the problem of complement activation. Moreover, decorin is a naturally occurring **proteoglycan** found in tissues throughout the body that exhibits good C1 complex inhibition. Decorin may in fact be involved in the. . .

SUMM Based upon these observations the inventors propose that other members of the decorin family of **proteoglycans**, including biglycan, lumican, and perhaps even fibromodulin. These **proteoglycans** demonstrate homology in the structure of their core proteins which are comprised predominantly of tandemly repeated segments 24 residues in. . .

SUMM . . . equally effective as naturally isolated decorin, so long as it is produced in a host cell that will produce the **proteoglycan** in a biologically active form. The decorin cDNA has been isolated and characterized, and can be employed to prepare recombinant. . .

DETD . . . of C1, thus, their biological relevance is uncertain. Recently, we have been investigating the interaction of C1q with a matrix **proteoglycan** called decorin.

DETD Decorin is a dermatan-sulfate **proteoglycan** which is widely distributed as an extracellular matrix component of numerous tissues including articular cartilage, bone, skin, adventitia of arterial. . . residues in length which contain leucine residues in conserved positions (48). Similar leucine-rich motifs have been detected in other matrix **proteoglycans** such as biglycan (49), lumican (50), and fibromodulin (51), as well as in several other proteins of diverse origin and. . .

DETD . . . decorin is concentrated at the surfaces of articular cartilage as determined by immunohistochemical studies (44), and that loss of stainable **proteoglycans** at the articular surface is the earliest histological change observed in rat models of collagen induced arthritis (64,65) suggesting that the surface **proteoglycans** may serve a protective role. Though this is an interesting possibility, at this time the physiologic significance the binding of. . .

DETD The preparation of an aqueous composition that contains a protein or **proteoglycan** as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid. . .

DETD A **proteoglycan** can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts. . .

DETD . . . as previously described, incorporated herein by reference (27). The inventors prepared core protein of decorin by digestion of the intact **proteoglycan** with chondroitinase ABC according to the manufacturer's instructions. Briefly, 2 ml of a 2 mg/ml solution of decorin in 0.1M. . .

DETD . . . (76) and included herein by reference. Gels were stained for 30 minutes with solutions of 0.25% Coomasie brilliant blue, 7% **acetic acid** and 50% methanol and destained with solutions of 10% methanol and 10% **acetic acid**. Alternatively, gels were silver stained as previously described by Goldman et al (106) and included herein by reference.

DETD . . . shows that neither the purified core protein, nor the isolated GAG chains of decorin inhibited C1 indicating that the intact **proteoglycan** is necessary for functional activity.

DETD . . . as described by Choi et al. (77). Briefly, a mixture of decorin (DSPG-II) and biglycan (DSPG-I) will be isolated from **cartilage** by dissociative **extraction** with 4M guanidine hydrochloride

TI Methods for the inhibition of complement activation
IN Krumdieck, Richard, Birmingham, AL, United States
Hook, Magnus A. O., Houston, TX, United States
Volanakis, John E., Birmingham, AL, United States
PA University of Alabama at Birmingham Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5650389 19970722
AI US 1993-25357 19930301 (8)
DT **Utility**
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Gambel, Phillip
LREP Adler, Benjamin Aaron
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1909
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Decorin, a small collagen-binding dermatan sulfate **proteoglycan** , is widely distributed as a component of extracellular matrices. Using a solid phase binding assay, the inventors demonstrated that decorin bound C1q at physiologic pH and ionic strength. The interaction did not require divalent cations and was time and temperature dependent reaching equilibrium in 4 hours at 37° C. Binding was specific and saturable with an apparent dissociation constant of 7.6+10.sup.-9 M. Decorin was shown to bind pepsin-derived fragments containing the collagenous domain of C1q and collagenase-derived fragments containing the globular domain of C1q. Since these fragments share a short sequence of amino acids, this finding suggests that decorin binds to a region of C1q located near the junction of the two domains. Competition studies using purified preparations of the decorin core protein and the glycosaminoglycan chains showed that only the former inhibited binding of decorin to C1q indicating that the interaction is mediated by the decorin core protein. Decorin was shown to inhibit the hemolytic activity of purified C1 as well as C1 in normal human serum. Approximately 50% inhibition was observed at a decorin concentration of 2 μg/ml. Inhibition was not observed if C1 was bound to antigen-complexed antibody. Furthermore, neither the core protein, nor the glycosaminoglycan chain of decorin inhibited C1 indicating that the intact **proteoglycan** is necessary for functional activity. These studies therefore demonstrate the usefulness of decorin and related **proteoglycans** in suppression of complement activation of the immune system.
DT **Utility**
AB Decorin, a small collagen-binding dermatan sulfate **proteoglycan** , is widely distributed as a component of extracellular matrices. Using a solid phase binding assay, the inventors demonstrated that decorin. . . to antigen-complexed antibody. Furthermore, neither the core protein, nor the glycosaminoglycan chain of decorin inhibited C1 indicating that the intact **proteoglycan** is necessary for functional activity. These studies therefore demonstrate the usefulness of decorin and related **proteoglycans** in suppression of complement activation of the immune system.
SUMM It is a further object to provide for the development of **proteoglycan** coatings for bio-materials such as plastic hemodialysis tubing or tubing used to achieve extracorporeal circulation during cardiac surgery, which inhibits. . .
SUMM . . . present invention is directed to these and other objects, and arises out of the inventors' discovery and demonstration that the

L15 ANSWER 9 OF 20 USPATFULL on STN
AN 97:98814 USPATFULL
TI Meniscal augmentation device
IN Li, Shu-Tung, Oakland, NJ, United States
Stone, Kevin R., Mill Valley, CA, United States
PA ReGen Biologics, Inc., Redwood City, CA, United States (U.S. corporation)
PI US 5681353 19971028
AI US 1994-250008 19940527 (8)
RLI Continuation-in-part of Ser. No. US 1994-232743, filed on 25 Apr 1994 which is a continuation-in-part of Ser. No. US 1991-809003, filed on 17 Dec 1991, now patented, Pat. No. US 5306311 which is a continuation-in-part of Ser. No. US 1990-520027, filed on 7 May 1990, now patented, Pat. No. US 5108438 which is a continuation-in-part of Ser. No. US 1989-317951, filed on 2 Mar 1989, now patented, Pat. No. US 5007934 which is a continuation-in-part of Ser. No. US 1987-75352, filed on 20 Jul 1987, now patented, Pat. No. US 4880429
DT **Utility**
FS Granted
EXNAM Primary Examiner: Isabella, David
LREP Fish & Richardson P.C.
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1041
AB The present invention relates to a meniscal augmentation device of biocompatible and at least partially bioresorbable fibers for implantation into a segmental defect of a meniscus in a subject. Upon implantation, the composite of the device and the meniscus establishes a scaffold adapted for ingrowth of meniscal fibrochondrocytes. The invention further pertains to methods of fabricating and using the device.
DT **Utility**
DETD . . . that are newly synthesized and have not yet been incorporated into the stable fibrils. Salt also removes some glycoproteins and **proteoglycans** that are associated with collagen through electrostatic interactions. Other salts such as KCl and the like can be used as. . .
DETD The acid swelling may be conducted at a low pH in the presence of **acetic acid**, HCl, or the like. Like the alkali treatment, the acid swelling removes non-crosslinked glycoproteins and GAGs.
DETD . . . neutralized with HCl and the material is washed with water. The pH is then adjusted to 2.5-3.0 by adding concentrated **acetic acid** to a final concentration of 0.5M. The acid extraction is continued for 24 (±2) hours with agitation.
DETD The **cartilage** tissues are **extracted** twice each with 10 volumes of 4M Guanidine-HCl for 24 hours at 4° C. with stirring. The **extracted cartilage** tissues are washed with 10 volumes of distilled water to remove the guanidine-HCl.
DETD The **cartilage** tissues are **extracted** in 10 volumes of 1% Triton X-100 and 2M NaCl for 24 hours with stirring at 4° C.
DETD The **cartilage** tissues are **extracted** with 10 volumes of 4M guanidine-HCl for 24 hours at 4° C. and the extractant is discarded. The insoluble residues. . .

L15 ANSWER 10 OF 20 USPATFULL on STN
AN 97:63990 USPATFULL

inhibit invasion by vascularized mesenchyme," Lab Invest, 32: 217-222, 1975.

L15 ANSWER 8 OF 20 USPATFULL on STN
AN 1998:36125 USPATFULL
TI Meniscal augmentation device
IN Li, Shu-Tung, 1 Kiowa Ter., Oakland, NJ, United States 07436
Stone, Kevin R., 1 Throckmorton La., Mill Valley, CA, United States 94941
PI US 5735903 19980407
AI US 1995-457971 19950601 (8)
RLI Division of Ser. No. US 1994-250008, filed on 27 May 1994 which is a continuation-in-part of Ser. No. US 1994-232243, filed on 25 Apr 1994, now patented, Pat. No. US 5624463 which is a continuation-in-part of Ser. No. US 1991-809003, filed on 17 Dec 1991, now patented, Pat. No. US 5306311 which is a continuation-in-part of Ser. No. US 1990-520027, filed on 7 May 1990, now patented, Pat. No. US 5108438 which is a continuation-in-part of Ser. No. US 1989-317951, filed on 2 Mar 1989, now patented, Pat. No. US 5007934 which is a continuation-in-part of Ser. No. US 1987-75352, filed on 20 Jul 1987, now patented, Pat. No. US 4880429
DT **Utility**
FS Granted
EXNAM Primary Examiner: Isabella, David
LREP Fish & Richardson P.C.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 987
AB The present invention relates to a meniscal augmentation device of biocompatible and at least partially bioresorbable fibers for implantation into a segmental defect of a meniscus in a subject. Upon implantation, the composite of the device and the meniscus establishes a scaffold adapted for ingrowth of meniscal fibrochondrocytes. The invention further pertains to methods of fabricating and using the device.
DT **Utility**
DETD . . . that are newly synthesized and have not yet been incorporated into the stable fibrils. Salt also removes some glycoproteins and **proteoglycans** that are associated with collagen through electrostatic interactions. Other salts such as KCl and the like can be used as. . .
DETD The acid swelling may be conducted at a low pH in the presence of **acetic acid**, HCl, or the like. Like the alkali treatment, the acid swelling removes non-crosslinked glycoproteins and GAGs.
DETD . . . neutralized with HCl and the material is washed with water. The pH is then adjusted to 2.5-3.0 by adding concentrated **acetic acid** to a final concentration of 0.5M. The acid extraction is continued for 24 (±2) hours with agitation.
DETD The **cartilage** tissues are **extracted** twice each with 10 volumes of 4M Guanidine-HCl for 24 hours at 4° C. with stirring. The **extracted cartilage** tissues are washed with 10 volumes of distilled water to remove the guanidine-HCl.
DETD The **cartilage** tissues are **extracted** in 10 volumes of 1% Triton X-100 and 2M NaCl for 24 hours with stirring at 4° C.
DETD The **cartilage** tissues are **extracted** with 10 volumes of 4M guanidine-HCl for 24 hours at 4° C. and the extractant is discarded. The insoluble residues. . .

loss of **proteoglycans** and an increase in water content are soon observed. The normal white, glistening appearance of the cartilage changes to a. . .

DETD . . . colony stimulating factor (GM-CSF), TNF, IL-1, IL-2, or other inflammatory cytokines. IL-1 has been shown to decrease the synthesis of **proteoglycans** and collagens type II, IX, and XI (Tyler et al., 1985, Biochem. J. 227:869-878; Tyler et al., 1988, Coll. Relat.. . . et al., 1988, J. Clin. Invest. 82:2026-2037; and Lefebvre et al., 1990, Biophys. Acta. 1052:366-372). TNF also inhibits synthesis of **proteoglycans** and type II collagen, although it is much less potent than IL-1 (Yaron, I., et al., 1989, Arthritis Rheum. 32:173-180;. . .

L15 ANSWER 7 OF 20 USPATFULL on STN
AN 1999:21711 USPATFULL
TI CXC chemokines as regulators of angiogenesis
IN Strieter, Robert M., Ann Arbor, MI, United States
Polverini, Peter J., Ann Arbor, MI, United States
Kunkel, Steven L., Ann Arbor, MI, United States
PA The Regent of the University of Michigan, Ann Arbor, MI, United States (U.S. corporation)
PI US 5871723 19990216
AI US 1995-468819 19950606 (8)
DT **Utility**
FS Granted
EXNAM Primary Examiner: Draper, Garnette D.
LREP Arnold, White & Durkee
CLMN Number of Claims: 29
ECL Exemplary Claim: 1
DRWN 17 Drawing Figure(s); 71 Drawing Page(s)
LN.CNT 6055
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are various discoveries concerning the angiogenic and angiostatic properties of the CXC chemokines, including the finding that the ELR motif controls the ability of these molecules to induce angiogenesis. Aspects of the invention include, for example, the identification of IP-10, MIG and certain IL-8 analogues as angiostatic agents, and their use in inhibiting angiogenesis in various systems.
DT **Utility**
DETD . . . is followed by epidermal regeneration and production of basement membrane extracellular constituents (fibronectin, type IV and VII collagen, heparin sulfate **proteoglycans**, and laminin) that provides the integrity of the epidermal to dermal structures (Davidson, 1992).
DETD . . . where they observed that hyaline cartilage was particularly resistant to vascular invasion. They reported that a heat labile guanidium chloride **extract** prepared from **cartilage** contained an inhibitor of neovascularization. Later Brem and Folkman (1975) and their co-workers Lee and Langer (1983) showed that a. . .
DETD . . . described (Koch et al., 1986). Nucleopore chemotaxis membranes (5 micron pore size) were prepared by soaking them sequentially in 3% **acetic acid** overnight and for 2 hr. in 0.1 mg/ml gelatin. Membranes were rinsed in sterile water, dried under sterile air, and. . .
DETD Eisenstein et al., "The resistance of certain tissues to invasion III. **Cartilage extracts** inhibit the growth of fibroblasts and endothelial cells in culture," Am. J. Pathol., 81: 337-347, 1975.
DETD Sorgente et al., "The resistance of certain tissues to invasion. II. Evidence for **extractable** factors in **cartilage** which

CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN 15 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2084
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to the isolation and use of pre-chondrocytes from the umbilical cord, specifically from Wharton's jelly, that give rise to chondrocytes which produce cartilage. The isolated pre-chondrocytes, or the chondrocytes to which they give rise, can be mitotically expanded in culture and used in the production of new cartilage tissue for therapeutic use. "Banks" of pre-chondrocytes or chondrocytes can be stored frozen, and thawed and used to produce new cartilage tissue as needed.

DT **Utility**

SUMM . . . pRb in rat liver epithelial cells (Whitson and Itakura, 1992, J. Cell. Biochem. 48:305-315); stimulates the production of BFGF binding **proteoglycans** (Nugent and Edelman, 1992, J. Biol. Chem. 267:21256-21264); modulates phosphorylation of the EGF receptor and proliferation of epidermoid carcinoma cells. . .

SUMM Insulin alone is much less potent than insulin-like growth factor (IGF-I) in stimulating collagen matrix synthesis. Insulin, however, enhances **proteoglycan** synthesis in the presence of a low concentration of serum (1%). IGF-I, previously designated somatomedin c, is a potent inducer of collagen and **proteoglycan** synthesis in vitro. (Lindahl et al., 1987, J. Endocrinol. 115:263-271; Markower et al., 1989, Cell. Biol. Int. Rep. 13:259-270).

SUMM . . . than IGF-I in stimulating clonal growth in fetal cells, whereas IGF-I is more effective on adult chondrocytes. IGF-II can stimulate **proteoglycan** synthesis, but, like insulin, is much less effective than IGF-I (McQuillan et al., 1986, Biochem. J. 240:423-430).

SUMM Epidermal growth factor (EGF) alone has no effect on chondrocyte proliferation. Together with insulin, EGF synergistically stimulates **proteoglycan** synthesis and induces proliferation of chondrocytes. (Osborn et al., 1989, J. Orthop. Res. 7:35-42). Basic fibroblast growth factor (bFGF) inhibits **proteoglycan** synthesis in fetal articular cartilage (Hamerman et al., 1986, J. Cell. Physiol. 127:317-322), but appears to function additively with IGF-I in adult articular cartilage and stimulates **proteoglycan** synthesis (Osborn, K. D., et al., 1989, J. Orthop. Res. 7:35-42). Platelet-derived growth factor (PDGF) also enhances **proteoglycan** synthesis (Prins et al., 1982, Arthritis Rheum. 25:1228-1238). Certain bone morphogenic proteins (BMPs) stimulate chondrogenesis and promote cartilage production (Inada. . .

SUMM In a further embodiment of the invention, extracellular matrix is **extracted** from new **cartilage** tissue produced by the cells of the invention, and is further processed to a formulation that is useful for the. . .

DETD . . . differs according to type; however, the general composition of cartilage comprises chondrocytes surrounded by a dense ECM consisting of collagen, **proteoglycans** and water. Several types of cartilage are recognized in the art, including, for example, hyaline cartilage, articular cartilage, costal cartilage,. . .

DETD . . . the matrix. For example, prior to inoculation with the cells of the invention, nylon matrices could be treated with 0.1M **acetic acid** and incubated in polylysine, PBS, and/or collagen to coat the nylon. Polystyrene could be similarly treated using sulfuric acid. Where. . .

DETD . . . are immobilized suffer relatively quickly in a number of respects. The metabolic activity of chondrocytes appears affected as

continuation-in-part of Ser. No. US 1990-520027, filed on 7 May 1990, now patented, Pat. No. US 5108438 which is a continuation-in-part of Ser. No. US 1989-317951, filed on 2 Mar 1989, now patented, Pat. No. US 5007934 which is a continuation-in-part of Ser. No. US 1987-75352, filed on 20 Jul 1987, now patented, Pat. No. US 4880429

DT **Utility**
FS Granted
EXNAM Primary Examiner: Isabella, David J.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1047
AB The present invention relates to a meniscal augmentation device of biocompatible and at least partially bioresorbable fibers for implantation into a segmental defect of a meniscus in a subject. Upon implantation, the composite of the device and the meniscus establishes a scaffold adapted for ingrowth of meniscal fibrochondrocytes. The invention further pertains to methods of fabricating and using the device.
DT **Utility**
DETD . . . that are newly synthesized and have not yet been incorporated into the stable fibrils. Salt also removes some glycoproteins and **proteoglycans** that are associated with collagen through electrostatic interactions. Other salts such as KCl and the like can be used as. . .
DETD The acid swelling may be conducted at a low pH in the presence of **acetic acid**, HCl, or the like. Like the alkali treatment, the acid swelling removes non-crosslinked glycoproteins and GAGs.
DETD . . . neutralized with HCl and the material is washed with water. The pH is then adjusted to 2.5-3.0 by adding concentrated **acetic acid** to a final concentration of 0.5M. The acid extraction is continued for 24 (±2) hours with agitation.
DETD The **cartilage** tissues are **extracted** twice each with 10 volumes of 4 M Guanidine-HCl for 24 hours at 4° C. with stirring. The **extracted cartilage** tissues are washed with 10 volumes of distilled water to remove the guanidine-HCl.
DETD The **cartilage** tissues are **extracted** in 10 volumes of 1% Triton X-100 and 2 M NaCl for 24 hours with stirring at 4° C.
DETD The **cartilage** tissues are **extracted** with 10 volumes of 4 M guanidine-HCl for 24 hours at 4° C. and the extractant is discarded. The insoluble. . .

L15 ANSWER 6 OF 20 USPATFULL on STN
AN 1999:75559 USPATFULL
TI Production of cartilage tissue using cells isolated from Wharton's jelly
IN Purchio, Anthony F., La Jolla, CA, United States
Naughton, Brian A., El Cajon, CA, United States
San Roman, Julia, San Diego, CA, United States
PA Advanced Tissue Science, Inc., La Jolla, CA, United States (U.S. corporation)
PI US 5919702 19990706
AI US 1996-735620 19961023 (8)
DT **Utility**
FS Granted
EXNAM Primary Examiner: Stanton, Brian R.; Assistant Examiner: Clark, Deborah J. R.
LREP Pennie & Edmonds llp

showing a higher **proteoglycan** concentration in the NP than the AF (26).

DETD . . . NP there was a significant trend towards lower concentrations of type II collagen in specimens with a low concentration of **proteoglycan** (r=0.78, p<0.05). The results show that although these intervertebral discs all had a normal gross morphology (see above), some of them nevertheless exhibit biochemical signs of matrix deterioration, demonstrated as a loss of **proteoglycan** accompanied by a loss of type II collagen in the NP.

DETD . . . in their gross morphology or magnetic resonance imaging profiles have shown that damage to the disc correlates with a decreased **proteoglycan** concentration (30,31). There was either no correlation with total collagen, measured as hydroxyproline, or possibly an increased collagen concentration with. . .

DETD We show that in the NP a loss of **proteoglycans** is associated with a loss of type II collagen in discs with normal gross morphology. These changes are indicative of. . . would be expected to be detrimental to disc function. Indeed, Pearce et al (30) have reported that a decrease in **proteoglycan** content accompanies disc degeneration and may precede overt morphological damage. Similarly, it is likely that the increased level of type. . .

DETD TABLE VII

The inhibition observed in **cartilage extracts** is lost on treatment with Clostridial collagenase [α1(II)-CB11B] by immunoassay (μg/ml)

Digested

Digested sample treated

Proteinase sample treated with Tris/CaCl.sub.2. . .

DETD 9. Rizkalla, G., Reiner, A., Bogoch, E. and Poole, A. R. (1992) Studies of the articular cartilage **proteoglycan** aggrecan in health and osteoarthritis. Evidence for molecular heterogeneity and extensive molecular changes in disease. J. Clin. Invest. 90:2268-2277.

DETD 17. Schmidt, M. B., Mow, V. C., Chun, L. E. and Eyre, D. R. (1990). Effects of **proteoglycan** extraction on the tensile behaviour of articular cartilage. J. Orthop. Res. 8:353-363.

DETD 36. McDevitt, C. A.: **Proteoglycans** of the intervertebral disc. The biology of the intervertebral disc. Vol. 1. Edited by P. Ghosh, Florida, CRC Press Inc.,. . .

DETD 43. Poole, A. R., Pidoux, I., Reiner, A., Tang, L- H., Choi, H., Rosenberg, L.: Localization of **proteoglycan** monomer and link protein in the matrix of bovine articular cartilage. J. Histochem. Cytochem. 28:621-635, (1980).

L15 ANSWER 5 OF 20 USPATFULL on STN

AN 2000:37168 USPATFULL

TI Meniscal augmentation device

IN Li, Shu-Tung, Oakland, NJ, United States
Stone, Kevin R., Mill Valley, CA, United States

PA ReGen Biologics, Inc., Franklin Lakes, NJ, United States (U.S. corporation)

PI US 6042610 20000328

AI US 1998-28284 19980224 (9)

RLI Continuation of Ser. No. US 1995-457971, filed on 1 Jun 1995, now patented, Pat. No. US 5735903 which is a division of Ser. No. US 1994-250088, filed on 27 May 1994, now patented, Pat. No. US 5479033 which is a continuation-in-part of Ser. No. US 1991-809003, filed on 17 Dec 1991, now patented, Pat. No. US 5306311 which is a

DETD Bovine native type II collagen was initially dissolved in 0.5M **acetic acid** and then diluted to a final concentration of 0.5 mg/ml in 100 mM Tris-HCl, pH 7.6, containing 10 mM CaCl.sub.2. . .

DETD . . . was mixed with 100 µl of the 1 mg/ml proteinase K solution and incubated and then inhibited as described for **cartilage** residues. **Extracts** were stored at 4° C. prior to assay for denatured collagen.

DETD . . . and OA cartilage specimens are shown in Table V. Previously, cartilage from these same sites have been analyzed for the **proteoglycan** aggrecan (9).

DETD . . . (II)-chain and α.sub.1 (II)-CB11B peptide were calculated as 98,291 and 2,231 respectively. Therefore, the µg/mg concentration of type II collagen **extracted** from **cartilage** was calculated by multiplying the concentration of extracted α.sub.1 (II)-CB11B by a factor of 44.

DETD It was important to demonstrate that inhibition observed in the al (II)-CB11B ELISA by α-chymotrypsin and proteinase K **extracts** of human **cartilage** was due entirely to the α.sub.1 (II)-CB11B epitope. Since the epitope is destroyed by treatment with clostridial collagenase (see above. . .

DETD . . . to the other, 5 mM CaCl.sub.2 only. A control tube contained collagenase in Tris with 5 mM CaCl.sub.2 but no **cartilage extract**. All tubes were incubated overnight at 37° C. and the collagenase activity was inhibited with EDTA as described above. The. . .

DETD . . . of immunoreactivity in each case (Table VII), demonstrating the specificity of the assay for the α.sub.1 (II)-CB11B epitope in the **cartilage extracts**.

DETD Guanidinium Chloride **Extracts** of OA **Cartilage**

DETD In order to determine if the type II collagen **extracted** from **cartilage** by α-chymotrypsin was mostly derived from fibril-associated, cross-linked collagen or from non-fibrillar, non-cross-linked α-chains, a comparison was made of extraction. . .

DETD **Extraction** of **Cartilage** Plugs with Guanidinium Chloride to Determine the Content of Non-Cross-Linked Collagen

DETD . . . seen as a loss of disc height (27,28). Histological changes have also been observed, including a loss of staining for **proteoglycan** and increased damage to collagen fibrils (29). However, biochemical analyses of these changes have been limited to a few studies in which total content of water, collagen and **proteoglycan** (31,31), or the relative proportion (32) and distribution (33) of different collagens, have been measured. In human intervertebral disc, the. . .

DETD . . . of the α.sub.1 (II)-CB11B epitope and of hydroxyproline. A second, adjacent block was used for the extraction and assay of **proteoglycans** and the third block was prepared for immunohistochemical analysis of denatured type II collagen.

DETD Extraction and Assay of **Proteoglycans**

DETD Significance of differences in the content of collagen, hydroxyproline and **proteoglycan** in the AF and NP compared to paired cartilage from each individual case were treated using the 2-tailed paired t-test.. . .

DETD Contents of Type II Collagen Relative to **Proteoglycans**

DETD The **proteoglycan** content, measured as GAG and expressed as µg/mg wet weight of tissue, was significantly higher in the NP than in. . . were 48.4(13.3) µg/mg, 58.3(12.0) µg/mg and 80.2(19.5) µg/mg respectively. This data is in agreement with previous studies

DETD Sorgente et al., "The resistance of certain tissues to invasion. II. Evidence for **extractable** factors in **cartilage** which inhibit invasion by vascularized mesenchyme," Lab Invest, 32:217-222, 1975.

L15 ANSWER 4 OF 20 USPATFULL on STN
AN 2000:138066 USPATFULL
TI Immunoassays for the measurement of collagen denaturation and cleavage in cartilage
IN Poole, Anthony Robin, Baie d'Urfe, Canada
Hollander, Anthony Peter, Greystones, United Kingdom
Billinghurst, R. Clark, Fort Collins, CO, United States
PA Shriners Hospitals for Children, Tampa, FL, United States (U.S. corporation)
PI US 6132976 20001017
AI US 1998-10999 19980122 (9)
RLI Continuation-in-part of Ser. No. US 1995-448501, filed on 17 Jul 1995, now abandoned which is a continuation of Ser. No. US 1992-984123, filed on 4 Dec 1992, now abandoned
DT **Utility**
FS Granted
EXNAM Primary Examiner: Stucker, Jeffrey
LREP Foley & Lardner
CLMN Number of Claims: 37
ECL Exemplary Claim: 1
DRWN 30 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 3018
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for detecting cartilage degradation in a biological sample by identifying the presence of unwound type II collagen in the biological sample, said method comprising:

contacting the biological sample with a monoclonal antibody which does not bind to native helical collagen but which does bind to an epitope on unwound type II collagen chains or fragments thereof, wherein said epitope has the following sequence (SEQ ID NO: 4):

A-P(OH)-G-E-D-G-R-P(OH)-G-P-P(OH)-G-P; and

detecting the presence of the bound monoclonal antibody. The present invention also relates to a method for detecting collagenase induced cartilage degradation in a biological sample by identifying the presence of an epitope on type II collagen which only becomes detectable following cleavage of said collagen by collagenase, said method comprising:

contacting the biological sample with a monoclonal antibody which binds to said epitope on type II collagen chains or fragments thereof containing said epitope; and

detecting the presence of said monoclonal antibody bound to the type II collagen and fragments.
DT **Utility**
SUMM . . . a tightly wound triple helix. In articular cartilage, type II collagen fibrils are responsible for the tensile strength whereas the **proteoglycans** provide the compressive stiffness necessary for normal articulation and function. The precise mechanisms by which these connective tissue components are. . .

(5 micron pore size) were prepared by soaking them sequentially in 3% **acetic acid** overnight and for 2 hr. in 0.1 mg/ml gelatin. Membranes were rinsed in sterile water, dried under sterile air, and. . .

DETD [0665] Eisenstein et al., "The resistance of certain tissues to invasion III. **Cartilage extracts** inhibit the growth of fibroblasts and endothelial cells in culture," Am. J. Pathol., 81:337-347, 1975.

DETD [0838] Sorgente et al., "The resistance of certain tissues to invasion. II. Evidence for **extractable** factors in **cartilage** which inhibit invasion by vascularized mesenchyme," Lab Invest, 32:217-222, 1975.

L15 ANSWER 3 OF 20 USPATFULL on STN
AN 2002:325686 USPATFULL
TI CXC chemokines as regulators of angiogenesis
IN Strieter, Robert M., Ann Arbor, MI, United States
Polverini, Peter J., Ann Arbor, MI, United States
Kunkel, Steven L., Ann Arbor, MI, United States
PA The Regents of the University of Michigan, Ann Arbor, MI, United States (U.S. corporation)
PI US 6491906 B1 20021210
AI US 1998-213383 19981209 (9)
RLI Division of Ser. No. US 1995-468819, filed on 6 Jun 1995, now patented, Pat. No. US 5871723
DT **Utility**
FS GRANTED
EXNAM Primary Examiner: Priebe, Scott D.; Assistant Examiner: Kavshal, Sumesh
LREP Williams, Morgan and Amerson
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN 174 Drawing Figure(s); 73 Drawing Page(s)
LN.CNT 6167
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are various discoveries concerning the angiogenic and angiostatic properties of the CXC chemokines, including the finding that the ELR motif controls the ability of these molecules to induce angiogenesis. Aspects of the invention include, for example, the identification of IP-10, MIG and certain IL-8 analogues as angiostatic agents, and their use in inhibiting angiogenesis in various systems.
DT **Utility**
DETD . . . is followed by epidermal regeneration and production of basement membrane extracellular constituents (fibronectin, type IV and VII collagen, heparin sulfate **proteoglycans**, and laminin) that provides the integrity of the epidermal to dermal structures (Davidson, 1992).

DETD . . . where they observed that hyaline cartilage was particularly resistant to vascular invasion. They reported that a heat labile guanidium chloride **extract** prepared from **cartilage** contained an inhibitor of neovascularization. Later Brem and Folkman (1975) and their co-workers Lee and Langer (1983) showed that a. . .

DETD . . . described (Koch et al., 1986). Nucleopore chemotaxis membranes (5 micron pore size) were prepared by soaking them sequentially in 3% **acetic acid** overnight and for 2 hr. in 0.1 mg/ml gelatin. Membranes were rinsed in sterile water, dried under sterile air, and. . .

DETD Eisenstein et al., "The resistance of certain tissues to invasion III. **Cartilage extracts** inhibit the growth of fibroblasts and endothelial cells in culture," Am . J. Pathol., 81:337-347, 1975.

various time points were assayed for amount of **proteoglycans** using the 1,9-dimethlymethylene blue (DMB) colorimetric assay (Parndale et al., Biochem. Biophys. Acta 883:173-177 (1992)). Chondroitin sulfate (Sigma) ranging from 0.0 to 5.0 μg was used to make the standard curve. To measure effects on **proteoglycan** synthesis, .sup.35S-sulfate (to a final concentration of 10 μCi/ml) (ICN Radiochemicals, Irvine, Calif.) was added to the cartilage explants at 48 hr. After an overnight incubation at 37° C., media was saved for measurements of nitric oxide or **proteoglycan** content. Cartilage pieces were washed two times using explant media. Digestion buffer containing 10 mM EDTA (pH 8.0), 0.1 M. . .

DETD . . . affects on cartilage matrix metabolism, porcine and human articular cartilage explants were treated with a range of IL-17F concentrations, and **proteoglycan** release and synthesis were measured. In both systems IL-17F induced significant cartilage matrix release (as shown in FIG. 48A and. . .

DETD . . . Tween-20. The sensor chip surface was regenerated between binding cycles by injection of a 25 uL aliquot of 0.1 M **acetic acid**, 0.2 M NaCl, pH 3 to elute non-covalently bound protein. Sensorgrams were evaluated according to a 1:1 binding model by. . .

L15 ANSWER 2 OF 20 USPATFULL on STN
AN 2003:44335 USPATFULL
TI Treatment of idiopathic pulmonary fibrosis using IP-10
IN Strieter, Robert M., Ann Arbor, MI, UNITED STATES
Kunkel, Steven L., Ann Arbor, MI, UNITED STATES
PA The Regents of The University of Michigan (U.S. corporation)
PI US 2003031645 A1 20030213
AI US 2002-104755 A1 20020321 (10)
RLI Continuation of Ser. No. US 1998-213383, filed on 9 Dec 1998, PENDING Division of Ser. No. US 1995-468819, filed on 6 Jun 1995, GRANTED, Pat. No. US 5871723
DT **Utility**
FS APPLICATION
LREP Shelley P.M. Fussey, WILLIAMS, MORGAN & AMERSON, P.C., Suite 250, 7676 Hillmont, Houston, TX, 77040
CLMN Number of Claims: 55
ECL Exemplary Claim: 1
DRWN 73 Drawing Page(s)
LN.CNT 6236
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are various discoveries concerning the angiogenic and angiostatic properties of the CXC chemokines, including the finding that the ELR motif controls the ability of these molecules to induce angiogenesis. Aspects of the invention include, for example, the identification of IP-10, MIG and certain IL-8 analogues as angiostatic agents, and their use in inhibiting angiogenesis in various systems.
DT **Utility**
DETD . . . is followed by epidermal regeneration and production of basement membrane extracellular constituents (fibronectin, type IV and VII collagen, heparin sulfate **proteoglycans**, and laminin) that provides the integrity of the epidermal to dermal structures (Davidson, 1992).

DETD . . . where they observed that hyaline cartilage was particularly resistant to vascular invasion. They reported that a heat labile guanidium chloride **extract** prepared from **cartilage** contained an inhibitor of neovascularization. Later Brem and Folkman (1975) and their co-workers Lee and Langer (1983) showed that a. . .

DETD . . . described (Koch et al., 1986). Nucleopore chemotaxis membranes

molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention. Further provided herein are methods for treating degenerative cartilaginous disorders and other inflammatory diseases.

DT **Utility**

DRWD . . . the indicated concentration (hatched) or IL-Ira (IL-1 receptor antagonist, R & D Systems, 1 ug/ml, for 72 hours). Release of **proteoglycans** (PG) into the media (top panel) indicates matrix breakdown. Matrix synthesis was determined by incorporation of .sup.35S-sulfate into the tissue. . .

DRWD . . . (L-NIO, Caymen Chemical, 0.5 mM). After 72 hours of treatment, media was assayed for nitrite (FIG. 41 part A.) and **proteoglycans** (Pgs) (FIG. 41 part B.). FIG. 41 part C. shows **proteoglycan** synthesis as determined by incorporation of .sup.35S-sulphate into the tissue.

DRWD [0132] FIG. 42 shows the effect of the inhibition of nitric oxide (NO) on IL-17 induced changes in **proteoglycan** (PG) metabolism. Articular cartilage explants were treated with IL-1α (5 ng/ml) alone (+) or with inhibitors of NOS (L-NIO or. . . R & D Systems, 1 μg/ml). After 72 hours of treatment, media was assayed for nitrite concentration and amount of **proteoglycans**. Matrix synthesis was determined by incorporation of .sup.35S-sulphate into the tissue.

DRWD . . . at 1% or 0.1% in the absence (leftmost 3 columns) or presence (rightmost 3 columns) of IL-1α (+) (10 ng/ml. **Proteoglycan** (PG) release and synthesis are shown as amount above control.

DRWD . . . concentrations (0.1, 1 or 10 nM) of IL-17F or IL-17. FIG. 48A shows the effect of IL-17F and IL-17 on **proteoglycan** breakdown, FIG. 48B shows the effect of IL-17F and IL-17 on **proteoglycan** synthesis, and FIG. 48C shows the effect of IL-17F and IL-17 on IL-6 production, respectively. Data represents the average of. . . concentrations (0.1, 1 or 10 nM) of IL-17F or IL-17. FIG. 48D shows the effect of IL-17F and IL-17 on **proteoglycan** breakdown, FIG. 48E shows the effect of IL-17F and IL-17 on **proteoglycan** synthesis, and FIG. 48F shows the effect of IL-17F and IL-17 on IL-6 production, respectively. Data represents the average of. . .

DETD . . . disorders that is characterized principally by the destruction of the cartilage matrix. Additional pathologies includes nitric oxide production, and elevated **proteoglycan** breakdown. Exemplary disorders encompassed within this definition, include, for example, arthritis (e.g., osteoarthritis, rheumatoid arthritis, psoriatic arthritis).

DETD . . . tubes contained media only. Media was harvested and changed at various timepoints (0, 24, 48, 72 hours) and assayed for **proteoglycan** content using the 1,9-dimethyl-methylene blue (DMB) colorimetric assay described in Farndale and Buttle, Biochem. Biophys. Acta, 883:173-177 (1985). After labeling. . . (overnight) with .sup.35S-sulfur, the tubes were weighed to determine the amount of tissue. Following an overnight digestion, the amount of **proteoglycan** remaining in the tissue as well as **proteoglycan** synthesis (.sup.35S-incorporation) is determined.

DETD [0680] IL-17 was observed to both increase the release of and decrease the synthesis of **proteoglycans** in both human and female pig articular **cartilage** explants (porcine **extracts** results are shown in FIG. 39). Moreover, this effect was additive to the effect observed from IL-1a. The effects of. . .

DETD [0695] To measure **proteoglycan** breakdown, media harvested at

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=> d que
L8              QUE  ABB=ON  PLU=ON  PROTEOGLYCAN AND ACETIC ACID AND CAR
                TILAGE(2A) EXTRACT?
L9           79 SEA L8
L10          77 DUP REM L9 (2 DUPLICATES REMOVED)
L11          75 SEA L10 AND P/DT
L12           2 SEA L10 NOT L11
L13          18 SEA L11 NOT PRD>07302001
L14           2 SEA L12 NOT PY>2001
L15          20 SEA L13 OR L14


=> d l15 bib ab kwic 1-20

L15  ANSWER 1 OF 20  USPATFULL on STN
AN      2003:288693  USPATFULL
TI      IL-17 homologous polypeptides and therapeutic uses thereof
IN      Chen, Jian, Princeton, NJ, UNITED STATES
        Filvaroff, Ellen, San Francisco, CA, UNITED STATES
        Fong, Sherman, Alameda, CA, UNITED STATES
        Goddard, Audrey, San Francisco, CA, UNITED STATES
        Godowski, Paul, Burlingame, CA, UNITED STATES
        Grimaldi, J. Christopher, San Francisco, CA, UNITED STATES
        Gurney, Austin, Belmont, CA, UNITED STATES
        Li, Hanzhong, San Mateo, CA, UNITED STATES
        Hillan, Kenneth, San Francisco, CA, UNITED STATES
        Hymowitz, Sarah G., San Francisco, CA, UNITED STATES
        Tumas, Daniel, Orinda, CA, UNITED STATES
        Starovasnik, Melissa A., San Francisco, CA, UNITED STATES
        VanLookeren, Menno, San Francisco, CA, UNITED STATES
        Vandlen, Richard, Hillsborough, CA, UNITED STATES
        Watanabe, Colin, Moraga, CA, UNITED STATES
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        Wood, William I., Hillsborough, CA, UNITED STATES
        Yansura, Daniel, Pacifica, CA, UNITED STATES
PA      Genentech, Inc., South San Francisco, CA, UNITED STATES, 94080 (U.S.
        corporation)
PI      US 2003203451      A1   20031030
AI      US 2003-458442     A1   20030610 (10)
RLI     Division of Ser. No. US 2001-874503, filed on 5 Jun 2001, PENDING
        Continuation-in-part of Ser. No. US 2001-816744, filed on 22 Mar 2001,
        GRANTED, Pat. No. US 6579520 Continuation-in-part of Ser. No. WO
        2001-US6520, filed on 28 Feb 2001, PENDING Continuation-in-part of Ser.
        No. US 2000-747259, filed on 20 Dec 2000, GRANTED, Pat. No. US 6569645
        Continuation-in-part of Ser. No. WO 2000-US23328, filed on 24 Aug 2000,
        PENDING
DT      Utility
FS      APPLICATION
LREP    GENENTECH, INC., 1 DNA WAY, SOUTH SAN FRANCISCO, CA, 94080
CLMN    Number of Claims: 60
ECL     Exemplary Claim: 1
DRWN    55 Drawing Page(s)
LN.CNT 8852
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      The present invention is directed to novel polypeptides having sequence
        identity with IL-17, IL-17 receptors and to nucleic acid molecules
        encoding those polypeptides. Also provided herein are vectors and host
        cells comprising those nucleic acid sequences, chimeric polypeptide
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